Available online at www.sciencedirect.com
SCIENCE DIRECT
Biochemical and Biophysical Research Communications 342 (2006) 66–72
BBRC
www.elsevier.com/locate/ybbrc

Cdx2 modulates proliferation in normal human intestinal epithelial crypt cells

Fabrice Escaffit, Frédéric Paré, Rémy Gauthier, Nathalie Rivard, François Boudreau, Jean-François Beaulieu *

CIHR Group on Functional Development and Physiopathology of the Digestive Tract, Département d'anatomie et de biologie cellulaire, Faculté de médecine et des sciences de la santé, Université de Sherbrooke, Sherbrooke, Que., Canada J1H 5N4

Received 24 January 2006
Available online 3 February 2006

Abstract

The homeobox gene Cdx2 is involved in the regulation of the expression of intestine specific markers such as sucrase-isomaltase and lactase-phlorizin hydrolase. Previous studies performed with immortalized or transformed intestinal cell lines have provided evidence that Cdx2 can promote morphological and functional differentiation in these experimental models. However, no data exist concerning the implication of this factor in normal human intestinal cell physiology. In the present work, we have investigated the role of Cdx2 in normal human intestinal epithelial crypt (HIEC) cells that lack this transcription factor. The establishment of HIEC cells expressing Cdx2 in an inducible manner shows that forced expression of Cdx2 significantly alters the proliferation of intestinal crypt cells and stimulates dipeptidylpeptidase IV expression but is not sufficient to trigger intestinal terminal differentiation. These observations suggest that Cdx2 requires additional factors to activate the enterocyte differentiation program in normal undifferentiated cells.

Keywords: Transcription factors; Cell proliferation; Cell differentiation; Intestinal cells

The crypt/villus functional unit is responsible for the continuous renewal of the intestinal epithelium. A dynamic system directs this phenomenon involving cell generation and migration from the stem cell population, located near the bottom of the crypts, to the extrusion of terminally differentiated cells at the tips of the villi [1]. Indeed, epithelial cells of the different compartments of the crypt–villus axis are characterized by differential properties in regard to cellular proliferation as well as differentiation. While the mechanisms controlling cell transition from one compartment to the next are complex and still incompletely understood, it is becoming more and more obvious that they involve specific transcription factors conferring compartment-specific gene expression along the crypt–villus axis [2].

The Cdx2 transcription factor belongs to the mammalian homeobox gene family related to the *Drosophila melanogaster* gene *caudal* [3,4] and has emerged as one of the major regulatory factors controlling intestinal cell differentiation [5–7]. The experimental evidence in support of a major role for Cdx2 includes the induction of cell differentiation in the immortalized rat intestinal epithelial cell line, IEC-6 [8], as well as the up-regulation of the expression of molecules involved in cell–cell or cell–substratum interactions such as LI-cadherin [9], E-cadherin, the integrin-β4 subunit [10], and claudin-2 [11] in various human colonic adenocarcinoma cell models. Mechanistically, Cdx2 has been described as functioning through physical interactions with other transcription factors, namely HNF-1, GATA-4, and C/EBP, to activate specific intestinal gene promoters such as those of sucrase-isomaltase and lactase-phlorizin hydrolase [12–16].

In the present work, we have investigated the involvement of Cdx2 in the differentiation of the normal human

* Corresponding author. Fax: +1 819 564 5320.
E-mail address: Jean-Francois.Beaulieu@USherbrooke.ca (J.-F. Beaulieu).

0006-291X/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2006.01.128

intestinal epithelial crypt cell model, HIEC. HIEC cells harbor specific features of intestinal crypt cells such as the expression of keratins 8, 18, and 20/21 [17], the crypt cell-specific marker MIM-1/39 [17], the integrin-α9 subunit [18], and an intestine-specific truncated form of the integrin-β4 subunit [19] as well as the brush border hydrolases aminopeptidase N and dipeptidylpeptidase IV [17,20,21]. However, HIEC cells appear to be unable to acquire the typical morphological or functional features of differentiated intestinal epithelial cells [17,20–22]. Consistent with these observations, we have found recently that HIEC cells lack HNF-1 and GATA-4 as well as Cdx2 [23]. Herein, our findings show that conditional expression of Cdx2 alters the proliferative ability of HIEC cells through an apparent cyclin D1 related mechanism while having only a minor influence on intestinal cell differentiation. Taken together, these results suggest that Cdx2 can regulate intestinal cell proliferation in a GATA-4 and HNF-1 independent manner but requires additional factors to activate the enterocyte differentiation program in normal cells.

Materials and methods

Cell culture. The HIEC-6 cell line was generated from a normal fetal human small intestine at mid-gestation [17]. These cells express typical features of the lower adult crypt region and are unable to differentiate [17–22,24]. HIEC cells may thus be considered to be intestinal lower crypt cells [20,21]. HIEC cells were used at passages 9–20 and were grown as previously described [17]. Caco2/15 cells were cultured in DMEM with 10% fetal bovine serum (Bio Media Canada Inc., Drummondville, QC), Glutamax and Hepes (GIBCO Invitrogen Corporation, Grand Island, NY) as previously described [25,26].

RT-PCR analysis and real-time PCR quantification. Total RNAs were extracted from HIEC or Caco2/15 cells using TriPure isolation reagent (Roche Diagnostics, Laval, QC). RNA integrity was verified on gel by ethidium bromide staining and quantities were determined spectrophotometrically. The reverse transcriptase Omniscript (Qiagen Inc., Mississauga, ON), 1 μM of oligo(dT)$_{12–18}$, 500 μM dNTP (Amersham Biosciences Corp.), and 9 U of RNAGuard (Amersham Biosciences Corp.) were added to 1 μg of total RNA for reverse-transcription for 1 h at 37 °C. Single-stranded cDNA was amplified by PCR in PCR buffer (Qiagen Inc.) containing 1 μM of both forward and reverse primers in the presence of 200 μM dNTPs and 2.5 U *Taq* (Qiagen Inc.) for 25 cycles of denaturation (1 min at 94 °C), annealing (1 min at the relevant temperature for each primer pair), and extension (1 min at 72 °C) in a PCR Express thermal cycler (Hybaid US, Franklin, MA). For human Cdx2 detection, we used the forward primer 5′-ACGTGAGCTACCTCCTGGAC-3′ and the reverse primer 5′-CAGGGACAGAGCCAGACACT-3′ which amplify a band of 888 bp at an annealing temperature (T_a) of 52 °C. Primers amplifying a region of 481 bp of sucrase-isomaltase (forward 5′-GAGG GATTGTAATTTCTCGTTCCC-3′ and reverse 5′-GGGGTAACCAT AAATGCTGG-3′, T_a: 56 °C) and 311 bp of dppIV (forward 5′-CCTT CTACTCTGATGAGTCACTGC-3′ and reverse 5′-GTGCCACTAAGC AGTTCCATCTTC-3′, T_a: 54 °C) were also used. β-actin amplification was used as a control to normalize the amounts of input RNA (forward 5′-TGACGGGGTCACCCACACTGTGCCCATCTA-3′ and reverse 5′-CTAGAAGCATTTGCGGTGGACGATGGAGGG-3′, T_a: 52 °C).

For quantitative evaluation of messenger levels, real-time experiments were carried out in an Mx3000P (Stratagene, Cedar Creek, TX). Each PCR contained 1 μl of RT product, 0.15 μM of each primer, 0.3× SYBR Green I, 0.8 mM dNTP, 2.5 mM $MgCl_2$, 2.5 U *Taq* DNA polymerase, and 1× buffer (Brilliant SYBR Green QPCR Core Reagent Kit, Stratagene). We used the same dppIV primers as for conventional RT-PCR experiments and S14 primers (forward 5′-GGCAGACCGAGATGAATCCT CA-3′ and reverse 5′-CAGGTCCAGGGGTCTTGGTCC-3′) for normalization. Following a 94 °C denaturation for 10 min, the reactions were cycled 40 times at 94 °C for 30 s, 58 °C for 1 min, and 72 °C for 1 min. Because SYBR Green binds to all double-stranded DNA, nonspecific products may be detected along with the target amplicon. To verify that only the specific product was amplified, a melting point analysis was done after the last cycle by cooling the samples to 55 °C and then increasing the temperature to 95 °C at a rate of 0.2 °C/s. A single product at a specific melting temperature was found for each gene target. Specific amplification was also confirmed by electrophoresis of the PCR products on agarose gel. Real-time PCR efficiencies were calculated from the slopes of the standard curves (threshold cycle (C_t) versus initial quantity of template) using the formula $E = 10^{(-1/\text{slope})}$ [27]. All samples were tested in triplicate and the experiment was repeated to evaluate variation between assays. Each run included a no-template control to test for contamination of assay reagents. Results were expressed in fold of variation versus Caco2/15 after correction by amplification efficiency of each target and normalization by the S14 signal for each sample, according to published mathematical studies [28].

Establishment of constitutive or inducible Cdx2 expressing HIEC cell lines by retroviral infections. Stable HIEC cell populations were established by a retroviral strategy because conventional transfection/selection procedures failed due to the limited life span (22–25 passages) of this normal cell line [23]. Viral strategies were thus used to improve the rate of gene transfer. For the constitutive Cdx2 expressing cell line, mouse Cdx2 cDNA was subcloned from pOPRSV1/Cdx2 (kindly provided by Dr. Traber, University of Pennsylvania) to pLHCX (BD Biosciences Clontech, Palo Alto, CA). pLHCX/Cdx2 was then used to produce virus in 293T cells cotransfected with the helper VPack-VSV and VPack-GP DNA vectors (Stratagene, La Jolla, CA). 293T cells ($5–8 \times 10^6$) were transfected for 4 h using Lipofectamine 2000 (GIBCO Invitrogen Corporation). Cell culture media (DMEM, GIBCO Invitrogen Corporation) were collected 48 h after infection, filtered through Millex-HA filters (25 mm, 0.45 μm; Millipore Corporation, Bedford, MA), and freshly plated subconfluent HIEC cells were then infected with the viral suspension containing 4 μg/ml of polybrene (hexadimethrine bromide, Sigma–Aldrich Canada Ltd, Oakville, ON). Cells were grown at 37 °C for 2 days before changing to medium containing the selection agent (10 μg/ml hygromycin, GIBCO Invitrogen Corporation). Selection was maintained until the cells reached confluence (about 20 days). Several stable HIEC^{Cdx2} cell populations were obtained as well as a control HIEC^{Empty} cell line selected after infection with a virus encoding for the empty pLHCX vector.

For the inducible approach, pTeton (BD Biosciences Clontech) was used to produce virus in 293T cells using the protocol described above. Selection of a stable HIEC^{Teton} cell population was achieved after culture with 250 μg/ml G418 (Bio Media Canada Inc.) and inducibility was verified by transitory transfection with pRevTRE/Cdx2 (see below), induction with increasing concentrations (0 to 5 μg/ml) of doxycycline (BD Biosciences Clontech) for 2 days of culture, harvesting of total proteins, and Western blotting of the lysates with an anti-Cdx2 antibody. The establishment of stable HIEC cell populations expressing Cdx2 in an inducible manner (HIEC^{IndCdx2} cells) was also accomplished by retroviral infection; mouse Cdx2 cDNA was subcloned into pRevTRE (BD Biosciences Clontech). Virus was produced and used to infect HIEC^{Teton} cells. Selection with 10 μg/ml hygromycin was then applied. The empty pRevTRE vector was used to produce virus for the establishment of the control HIEC^{IndEmpty} cell line. Thereafter, HIEC^{IndEmpty} and HIEC^{IndCdx2} cells were used in various experiments after culture under uninducing (0 μg/ml doxycycline) or optimally inducing (5 μg/ml) conditions and total RNA or protein was harvested at different times.

Western blot analysis. Proteins from HIEC cell lines were directly extracted in sample buffer containing 1% Triton, 150 mM sodium chloride, 200 μM sodium orthovanadate, 25 mM sodium fluoride, and 1 mM protease inhibitors (PMSF, aprotinin, and pepstatin) in 50 mM Tris/HCl buffer, pH 7.4. Sodium dodecyl sulfate (SDS)/12% PAGE and Western blotting were performed after addition of loading buffer containing 5% β-mercaptoethanol (Sigma–Aldrich Canada Ltd, Oakville,

ON). Separated proteins (50 μg/lane) were transferred onto nitrocellulose (Amersham Biosciences Corp, Baie d'Urfé, PQ) and membranes were blocked for 1 h at room temperature in PBS (pH 7.4) containing 5% Blotto and incubated with a primary antibody directed to Cdx2 (dilution 1/500, mouse monoclonal antibody from BioGenex, San Ramon, CA) and cyclin D1 (1/400, rabbit polyclonal antibody; Santa Cruz Biotechnology Inc.). An anti-cytokeratin18 antibody (clone CY90, 1/200; Sigma–Aldrich Canada Ltd) was used to ensure equivalent loadings. The membranes were then incubated with the anti-mouse or anti-rabbit secondary antibody conjugated to horseradish peroxidase and developed with the ECL Western blotting kit (Amersham Biosciences Corp.). Quantifications were done using Scion Image software (Scion Corp., Frederick, MA).

Morphological analysis. Monolayers of postconfluent HIEC^{IndEmpty} and HIEC^{IndCdx2} cells, grown in 35 mm dishes in OptiMEM with or without 5 μg/ml doxycycline, were observed under visible light with a microscope (DM; Leica) equipped with a cooled CCD camera (MicroMAX-5 MHz-1300Y, Princeton Instrument Inc.).

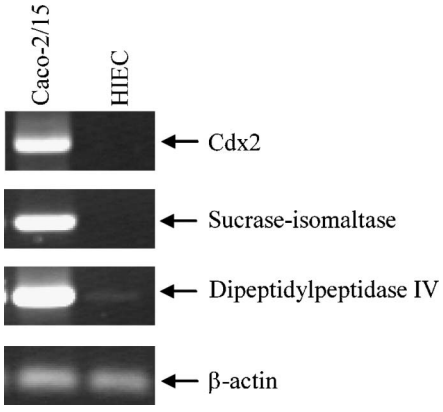

Fig. 1. Characterization of Cdx2, sucrase-isomaltase, and dipeptidylpeptidase IV expression in HIEC cells. One microgram of mRNA from Caco-2/15 or HIEC cells was reverse transcribed and assayed by PCR using primers for each cDNA. Primers for β-actin were used for normalization.

Cell proliferation assays. Inducible stable cell populations were seeded in 35 mm dishes with 2×10^5 cells per dish in complete OptiMEM with or without 5 μg/ml doxycycline. At various intervals between day 2 and 20 after seeding, three plates of each cell line were washed with PBS and the individual cell suspensions were prepared by trypsinization and counted with a Z1 Coulter Counter (Beckman Instruments Inc., Mississauga, ON).

Results

Differentiation marker expression is repressed in normal crypt-like HIEC cells

The HIEC-6 cell line was used as an experimental model for human intestinal crypt cells. HIEC cells have been previously characterized for their expression of keratins and intestinal cell markers typical of undifferentiated intestinal crypt cells [17,20]. As illustrated (Fig. 1), RT-PCR analysis revealed a low amount of dipeptidylpeptidase IV mRNA and a lack of sucrase-isomaltase mRNA of the HIEC cell line. Interestingly, the Cdx2 transcript, as for those encoding GATA-4 and HNF-1α [23], was lacking.

Establishment of stable HIEC cell lines expressing Cdx2

In order to analyze the role of the transcription factor Cdx2 in human intestinal crypt cell physiology, we used viral transduction strategies to establish Cdx2 expressing HIEC cell populations. In the first approach, we used the pLNCX2/Cdx2-encoded retrovirus to force constitutive expression of Cdx2 in HIEC cells. However, all attempts resulted in the generation of poorly growing cell populations (data not shown).

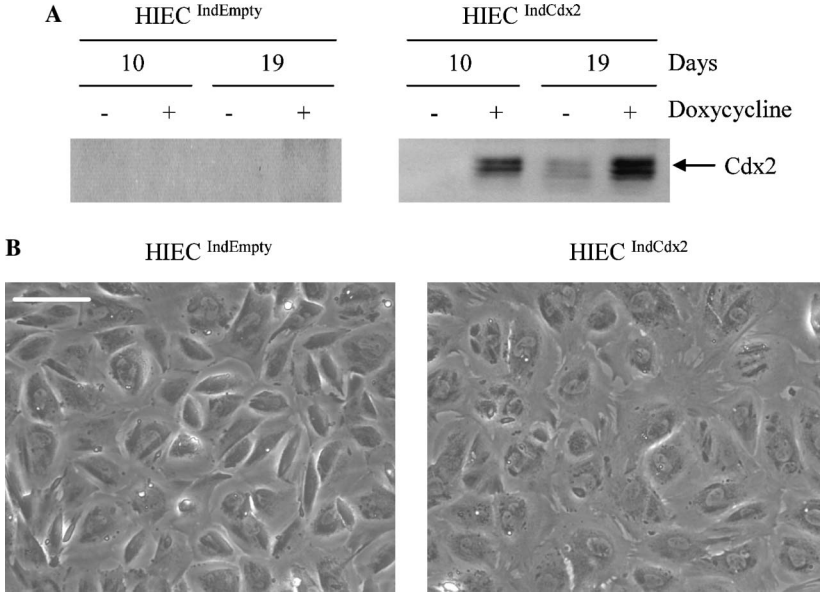

Fig. 2. Cdx2 immunodetection and light microscopy analysis of HIEC^{IndEmpty} and HIEC^{IndCdx2} cells. (A) Western blot was performed on 50 μg of protein samples from HIEC^{IndEmpty} and HIEC^{IndCdx2} cells grown ± 5μg/ml doxycycline and harvested after 10 and 19 days. (B) For light microscopy, cells were grown in 100 mm dishes in the presence of 5 μg/ml doxycycline and morphology was observed 5 days after confluence under phase contrast using a microscope equipped with a cooled CCD camera. Bar = 30 μm.

We then used the doxycycline-induced expression system as a second approach. This allowed the selection of stable HIEC^{IndCdx2} cell lines that expressed relatively high amounts of Cdx2 in an inducible manner in about 80% of cells (not shown). The optimal induction condition of 5μg/ml of doxycycline was determined from a Cdx2 inducibility curve of doxycycline from 0 to 10 μg/ml (data not shown). As illustrated in Fig. 2 with one representative HIEC^{IndCdx2} line, Cdx2 expression with doxycycline stimulation was found to be maintained over time and passage (Fig. 2A, right panel). A weak but persistent expression of Cdx2 was also detected in HIEC^{IndCdx2} even in the absence of doxycycline indicating some ''leakyness'' of the system (Fig. 2A, right panel). By contrast, HIEC^{IndEmpty} cells exhibited no Cdx2 expression (Fig. 2A, left panel).

Morphologically, doxycycline-treated HIEC^{IndEmpty} and HIEC^{IndCdx2} cells both displayed an epithelioid morphology at confluence (Fig. 2B, left and right panels, respectively) similar to the parental HIEC.

Modulation of HIEC proliferation and differentiation marker expression by Cdx2

We first investigated proliferation in the Cdx2-inducible HIEC cells. As shown in Fig. 3A, doxycycline-induced expression of Cdx2 in HIEC^{IndCdx2} cells resulted in a significant decrease (about 50%) in the proliferation rate as compared to the HIEC^{IndEmpty} control cell line treated with or without doxycycline. Interestingly, a significant reduction of the proliferation rate was also observed in the doxycy-

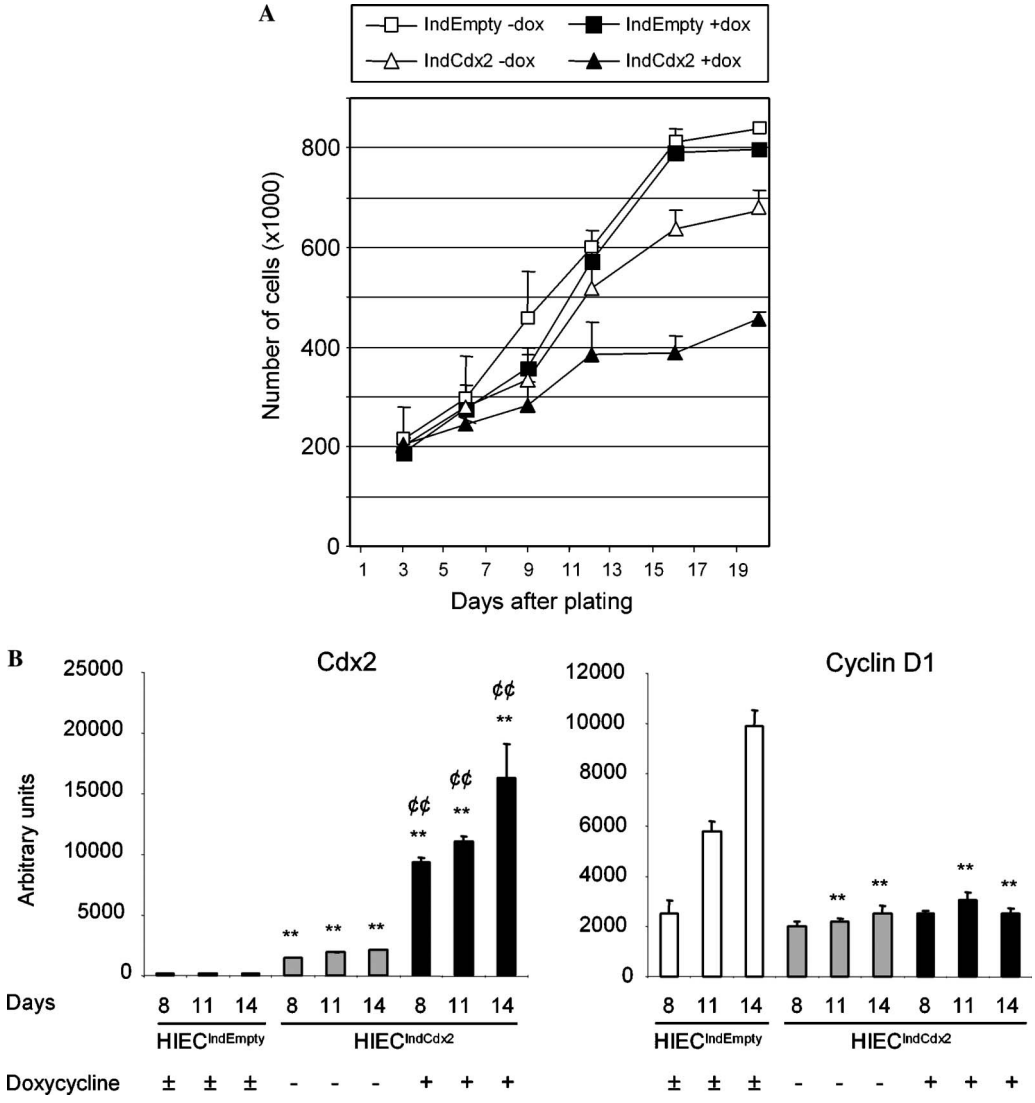

Fig. 3. Proliferation assay with HIEC inducible cell lines (A) and analysis of Cdx2 and cyclinD1 levels (B). HIEC^{IndEmpty} and HIEC^{IndCdx2} cells were seeded in 35 mm dishes at 2×10^5 cells per dish with or without 5 μg/ml doxycycline and kept in culture until day 20 after seeding (A). The medium was replaced every two days and the cells of three plates of each cell line were trypsinized and counted at different times. (B) The same protocol was used to culture inducible cells ± doxycycline and harvest total protein 8, 11, and 14 days after plating (exponential growth phase) for the analysis of Cdx2 and cyclin D1 expression by Western blot. After quantification, statistical analysis was performed to compare (a) HIEC^{IndCdx2} cells ± doxycycline with the control HIEC^{IndEmpty} population (**$p < 0.02$) and (b) doxycycline-induced HIEC^{IndCdx2} cells with the corresponding uninduced population (¢¢$p < 0.02$).

cline-uninduced HIEC^{IndCdx2} cells, suggesting that even the low expression of Cdx2 resulting from the leakyness of the inducible system can alter cell growth (Fig. 3A). Western blot analysis of growing cells (Fig. 3B) revealed that the expression of cyclin D1 (right panel) was significantly reduced in Cdx2 expressing HIEC cells at 11 and 14 days after plating. It is noteworthy that cyclin D1 expression was inhibited even when Cdx2 was expressed at low levels in the absence of doxycycline (Fig. 3B, left panel).

The effect of Cdx2 expression on differentiation was assessed by phase contrast and electron microscopy as well as by determining the expression of the intestinal cell markers dipeptidylpeptidase IV and sucrase-isomaltase. Doxycycline-treated HIEC^{IndCdx2} cells were not found to express any distinct features by either optical or electron microscopy that would indicate that the Cdx2 expressing cells had acquired a significantly more differentiated phenotype (Fig. 2B and data not shown). Measurement of dipeptidylpeptidase IV and sucrase-isomaltase transcript levels (Fig. 4) led to the same conclusion. Indeed, while a limited and statistically significant increase of dipeptidylpeptidase IV mRNA was observed in Cdx2 expressing cells

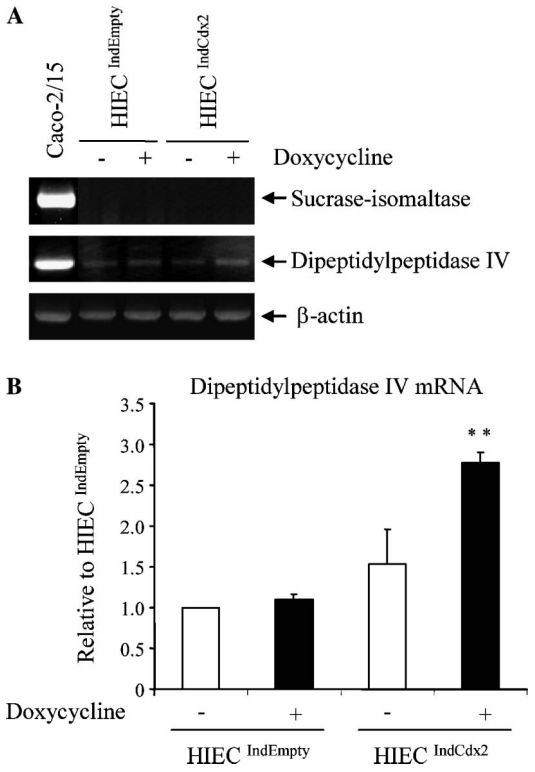

Fig. 4. Detection and quantification of sucrase-isomaltase and dipeptidylpeptidase IV expression by RT-PCR and real-time PCR in inducible HIEC cell lines ± doxycycline. One microgram of mRNA from each sample was reverse transcribed and assayed by PCR for both genes (A) or real-time PCR for dipeptidylpeptidase IV (B) using specific primers. Primers for β-actin and S14 were used for normalization. A Caco-2/15 reverse-transcribed sample was used as positive control. Statistical analysis was performed to compare dipeptidylpeptidase IV expression in the HIEC^{IndCdx2} cells ± doxycycline with the control HIEC^{IndEmpty} population (**$p < 0.02$).

(Figs. 4A and B), no induction of sucrase-isomaltase was obtained in response to the forced Cdx2 expression.

Discussion

The Cdx2 transcription factor has been previously described as being a regulator of differentiation in the immortalized rat intestinal epithelial cell line IEC-6 [8] and of gene expression in various colon carcinoma cell lines [9–11]. However, as shown herein, conditional expression of Cdx2 in intestinal cells lacking endogenous HNF-1 and GATA-4, such as the normal human epithelial crypt HIEC cells [23], was not sufficient to trigger the morphological and gene expression changes related to terminal enterocyte differentiation, although a modest increase in the expression of the intestinal cell marker dipeptidylpeptidase IV [17,21,29] was noted. Cdx2 expression did however significantly alter cell proliferation under a cyclin D1 related mechanism in normal intestinal cells. These results were obtained using mouse Cdx2 cDNA but the experiments performed with a human Cdx2 provided exactly the same results (data not shown). The data strengthen the idea that other transcription factors, such as HNF-1α and GATA-4, are required to cooperate with Cdx2 in order to trigger intestine specific gene expression, as demonstrated for sucrase-isomaltase [15]. In this context, the human HIEC cell line, used herein, appears fundamentally distinct from its rat counterpart IEC-6, which can be induced to differentiate by forcing Cdx2 expression [8]. Indeed, in contrast to HIEC cells [23], IEC-6 cells express endogenous levels of both HNF-1 and GATA-4 (Boudreau F., personal communication).

Used as a model to study the HNF-1- and GATA-4-independent effects of Cdx2 on intestinal cell physiology, forced expression of Cdx2 in HIEC cells revealed an inhibitory effect on cell proliferation. While consistent with previous observations performed in IEC-6 cells as well as in colon carcinoma cell lines [8,30–32], the results presented here suggest that this Cdx2 effect was independent of its cooperative role with HNF-1α and GATA-4. Interestingly, Cdx2 was found to be degraded when constitutively expressed in HIEC cells, supporting the existence of a regulatory mechanism controlling Cdx2 expression at the protein level [33,34]. This phenomenon was not observed in HIEC cells in which Cdx2 expression was controlled by the inducible system.

The mechanism by which Cdx2 inhibits HIEC cell proliferation was further investigated at the cellular level. Indeed, recent studies have reported that Cdx2 can inhibit colon cancer cell proliferation by either reducing β-catenin/TCF transcriptional activity [31,32] and/or by upregulating transcription of the $p21^{WAF1/CIP1}$ gene [30]. In HIEC cells, no inhibitory effect of Cdx2 expression was found on β-catenin/TCF transcriptional activity by using the canonical β-catenin/TCF reporter construct TOPFLASH (data not shown). However, Cdx2 expression resulted in a marked diminution of

cyclin D1 at the protein level. Inhibition of cyclin D1 expression has been previously reported in human colon cancer DLD1 and HCT116 cells in response to Cdx1 expression [35]. Interestingly, Cdx1 expression did reduce cyclin D1 promoter activity, suggesting that Cdx1 acts to diminish cyclin D1 gene transcription [35]. Considering the complementary and overlapping roles proposed for Cdx1 and Cdx2 [36], such a mechanism would be consistent with the cyclin D1 reduction observed at the protein level in HIEC cells in response to Cdx2. On the other hand, levels of the cyclin-dependent kinase inhibitor $p21^{Cip1}$ remained unchanged in HIEC cells expressing Cdx2 as compared to control cells (data not shown). In colon carcinoma cells, Cdx2 has been reported to transactivate the $p21^{Cip1}$ promoter in a p53-independent manner [30] while a transient and marked expression of $p21^{Cip1}$ has been associated with a p53-dependent irreversible growth arrest during the early stages of intestinal cell differentiation in the conditionally immortalized human intestinal cell line tsFHI [21,37]. While p53 is only detected at low levels in HIEC cells [21], the lack of effect of Cdx2 on the $p21^{Cip1}$ promoter could be, as for other promoters controlling intestinal genes (see above), ascribed to the lack of Cdx2-cooperating transcription factor(s) in HIEC cells. Because of the well-documented role of both Cdx2 [8–11] and $p27^{Kip1}$ [24,29,37] in intestinal cell differentiation, levels of $p27^{Kip1}$ and its structurally related form $p57^{Kip2}$ were analyzed but remained relatively low under all conditions (data not shown). It is noteworthy that subconfluent HIEC cells express very weak levels of both $p27^{Kip1}$ and $p57^{Kip2}$ relative to postconfluent HIEC or Caco-2 cells [24] suggesting that their role on cell growth inhibition of Cdx2-expressing HIEC cells is of limited impact.

Acknowledgments

The authors thank Dominique Jean for his technical assistance with the cloning and Elizabeth Herring for reviewing the manuscript. The work was supported by grants from the Canadian Institutes of Health Research (MOP-57727 and MGC-15186). J.F.B. holds a Canadian Research Chair in Intestinal Physiopathology. N.R. holds a Canadian Research Chair in Signaling and Digestive Physiopathology. F.B. is a chercheur-boursier of the Fonds de la recherche en santé du Québec.

References

[1] M.W. Babyatsky, D.K. Podolsky, Growth and development of the gastrointestinal tract, in: T. Yamada (Ed.), Textbook of Gastroenterology, J.B. Lippincott, Philadelphia, 1999, pp. 547–584.
[2] D.C. Rubin, Small intestine: anatomy and structural anomalies, in: T. Yamada (Ed.), Textbook of Gastroenterology, J.B. Lippincott, Philadelphia, 1999, pp. 1561–1583.
[3] P. Duprey, K. Chowdhury, G.R. Dressler, R. Balling, D. Simon, J.L. Guenet, P. Gruss, A mouse gene homologous to the Drosophila gene caudal is expressed in epithelial cells from the embryonic intestine, Genes Dev. 2 (1988) 1647–1654.
[4] R. James, J. Kazenwadel, Homeobox gene expression in the intestinal epithelium of adult mice, J. Biol. Chem. 266 (1991) 3246–3251.
[5] R.K. Montgomery, A.E. Mulberg, R.J. Grand, Development of the human gastrointestinal tract: twenty years of progress, Gastroenterology 116 (1999) 702–731.
[6] P.G. Traber, Development of the brushborder enzyme activities, in: I.R. Sanderson, W.A. Walker (Eds.), Development of the Gastrointestinal Tract, B.C. Decker, Hamilton, 2000, pp. 103–122.
[7] F. Beck, The role of Cdx genes in the mammalian gut, Gut 53 (2004) 1394–1396.
[8] E. Suh, P.G. Traber, An intestine-specific homeobox gene regulates proliferation and differentiation, Mol. Cell Biol. 16 (1996) 619–625.
[9] T. Hinoi, P.C. Lucas, R. Kuick, S. Hanash, K.R. Cho, E.R. Fearon, CDX2 regulates liver intestine-cadherin expression in normal and malignant colon epithelium and intestinal metaplasia, Gastroenterology 123 (2002) 1565–1577.
[10] O. Lorentz, I. Duluc, A.D. Arcangelis, P. Simon-Assmann, M. Kedinger, J.N. Freund, Key role of the Cdx2 homeobox gene in extracellular matrix-mediated intestinal cell differentiation, J. Cell Biol. 139 (1997) 1553–1565.
[11] T. Sakaguchi, X. Gu, H.M. Golden, E. Suh, D.B. Rhoads, H.C. Reinecker, Cloning of the human claudin-2 5′-flanking region revealed a TATA-less promoter with conserved binding sites in mouse and human for caudal-related homeodomain proteins and hepatocyte nuclear factor-1alpha, J. Biol. Chem. 277 (2002) 21361–21370.
[12] C. Mitchelmore, J.T. Troelsen, N. Spodsberg, H. Sjostrom, O. Noren, Interaction between the homeodomain proteins Cdx2 and HNF1alpha mediates expression of the lactase-phlorizin hydrolase gene, Biochem. J. 346 (Pt. 2) (2000) 529–535.
[13] R. Fang, N.A. Santiago, L.C. Olds, E. Sibley, The homeodomain protein Cdx2 regulates lactase gene promoter activity during enterocyte differentiation, Gastroenterology 118 (2000) 115–127.
[14] S.D. Krasinski, H.M. Van Wering, M.R. Tannemaat, R.J. Grand, Differential activation of intestinal gene promoters: functional interactions between GATA-5 and HNF-1 alpha, Am. J. Physiol. Gastrointest. Liver Physiol. 281 (2001) G69–G84.
[15] F. Boudreau, E.H. Rings, H.M. van Wering, R.K. Kim, G.P. Swain, S.D. Krasinski, J. Moffett, R.J. Grand, E.R. Suh, P.G. Traber, Hepatocyte nuclear factor-1 alpha, GATA-4, and caudal related homeodomain protein Cdx2 interact functionally to modulate intestinal gene transcription. Implication for the developmental regulation of the sucrase-isomaltase gene, J. Biol. Chem. 277 (2002) 31909–31917.
[16] L.J. Staloch, J.K. Divine, J.T. Witten, T.C. Simon, C/EBP and Cdx family factors regulate liver fatty acid binding protein transgene expression in the small intestinal epithelium, Biochim. Biophys. Acta 1731 (2005) 168–178.
[17] N. Perreault, J.F. Beaulieu, Use of the dissociating enzyme thermolysin to generate viable human normal intestinal epithelial cell cultures, Exp. Cell Res. 224 (1996) 354–364.
[18] N. Desloges, N. Basora, N. Perreault, Y. Bouatrouss, D. Sheppard, J.F. Beaulieu, Regulated expression of the integrin alpha9beta1 in the epithelium of the developing human gut and in intestinal cell lines: relation with cell proliferation, J. Cell Biochem. 71 (1998) 536–545.
[19] N. Basora, F.E. Herring-Gillam, F. Boudreau, N. Perreault, L.P. Pageot, M. Simoneau, Y. Bouatrouss, J.F. Beaulieu, Expression of functionally distinct variants of the beta(4)A integrin subunit in relation to the differentiation state in human intestinal cells, J. Biol. Chem. 274 (1999) 29819–29825.
[20] L.P. Pageot, N. Perreault, N. Basora, C. Francoeur, P. Magny, J.F. Beaulieu, Human cell models to study small intestinal functions: recapitulation of the crypt–villus axis, Microsc. Res. Tech. 49 (2000) 394–406.

[21] A. Quaroni, J.F. Beaulieu, Cell dynamics and differentiation of conditionally immortalized human intestinal epithelial cells, Gastroenterology 113 (1997) 1198–1213.
[22] N. Perreault, J.F. Beaulieu, Primary cultures of fully differentiated and pure human intestinal epithelial cells, Exp. Cell Res. 245 (1998) 34–42.
[23] F. Escaffit, F. Boudreau, J.F. Beaulieu, Differential expression of claudin-2 along the human intestine: implication of GATA-4 in the maintenance of claudin-2 in differentiating cells, J. Cell Physiol. 203 (2005) 15–26.
[24] C. Deschenes, A. Vezina, J.F. Beaulieu, N. Rivard, Role of p27(Kip1) in human intestinal cell differentiation, Gastroenterology 120 (2001) 423–438.
[25] J.F. Beaulieu, B. Nichols, A. Quaroni, Posttranslational regulation of sucrase-isomaltase expression in intestinal crypt and villus cells, J. Biol. Chem. 264 (1989) 20000–20011.
[26] P.H. Vachon, J.F. Beaulieu, Extracellular heterotrimeric laminin promotes differentiation in human enterocytes, Am. J. Physiol. 268 (1995) G857–G867.
[27] M.W. Pfaffl, G.W. Horgan, L. Dempfle, Relative expression software tool(REST)for group-wise comparison and statistical analysis of relative expression results in real-time PCR, Nucleic Acids Res. 30 (2002) e36.
[28] M.W. Pfaffl, A new mathematical model for relative quantification in real-time RT-PCR, Nucleic Acids Res. 29 (2001) e45.
[29] A. Quaroni, J.Q. Tian, P. Seth, C. Ap Rhys, p27(Kip1) is an inducer of intestinal epithelial cell differentiation, Am. J. Physiol. Cell Physiol. 279 (2000) C1045–C1057.
[30] Y.Q. Bai, S. Miyake, T. Iwai, Y. Yuasa, CDX2, a homeobox transcription factor, upregulates transcription of the p21/WAF1/CIP1 gene, Oncogene 22 (2003) 7942–7949.
[31] R.J. Guo, E. Huang, T. Ezaki, N. Patel, K. Sinclair, J. Wu, P. Klein, E.R. Suh, J.P. Lynch, Cdx1 inhibits human colon cancer cell proliferation by reducing beta-catenin/T-cell factor transcriptional activity, J. Biol. Chem. 279 (2004) 36865–36875.
[32] M.S. Keller, T. Ezaki, R.J. Guo, J.P. Lynch, Cdx1 or Cdx2 expression activates E-cadherin-mediated cell–cell adhesion and compaction in human COLO 205 cells, Am. J. Physiol. Gastrointest. Liver Physiol. 287 (2004) G104–G114.
[33] J. Boulanger, A. Vezina, S. Mongrain, F. Boudreau, N. Perreault, B.A. Auclair, J. Laine, C. Asselin, N. Rivard, Cdk2-dependent phosphorylation of homeobox transcription factor CDX2 regulates its nuclear translocation and proteasome-mediated degradation in human intestinal epithelial cells, J. Biol. Chem. 280 (2005) 18095–18107.
[34] C. Gespach, Crosstalk between the cell cycle regulator Cdk2 and the Cdx2 tumor suppressor at the proliferation-differentiation interface, Oncogene 24 (2005) 7953–7954.
[35] J. Lynch, M. Keller, R.J. Guo, D. Yang, P. Traber, Cdx1 inhibits the proliferation of human colon cancer cells by reducing cyclin D1 gene expression, Oncogene 22 (2003) 6395–6407.
[36] E. van den Akker, S. Forlani, K. Chawengsaksophak, W. de Graaff, F. Beck, B.I. Meyer, J. Deschamps, Cdx1 and Cdx2 have overlapping functions in anteroposterior patterning and posterior axis elongation, Development 129 (2002) 2181–2193.
[37] J.Q. Tian, A. Quaroni, Involvement of p21(WAF1/Cip1) and p27(Kip1) in intestinal epithelial cell differentiation, Am. J. Physiol. 276 (1999) C1245–C1258.