Targeted ablation of secretin-producing cells in transgenic mice reveals a common differentiation pathway with multiple enteroendocrine cell lineages in the small intestine

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SUMMARY

The four cell types of gut epithelium, enteroendocrine cells, enterocytes, Paneth cells and goblet cells, arise from a common totipotent stem cell located in the mid portion of the intestinal gland. The secretin-producing (S) cell is one of at least ten cell types belonging to the diffuse neuroendocrine system of the gut. We have examined the developmental relationship between secretin cells and other enteroendocrine cell types by conditional ablation of secretin cells in transgenic mice expressing herpes simplex virus 1 thymidine kinase (HSVTK). Ganciclovir-treated mice showed markedly increased numbers of apoptotic cells at the crypt-villus junction. Unexpectedly, ganciclovir treatment induced nearly complete ablation of enteroendocrine cells expressing cholecystokinin and peptide YY/glucagon (L cells) as well as secretin cells, suggesting a close developmental relationship between these three cell types. In addition, ganciclovir reduced the number of enteroendocrine cells producing gastrin inhibitory polypeptide, substance-P, somatostatin and serotonin. During recovery from ganciclovir treatment, the enteroendocrine cells repopulated the intestine in normal numbers, suggesting that a common early endocrine progenitor was spared. Expression of BETA2, a basic helix-loop-helix protein essential for differentiation of secretin and cholecystokinin cells was examined in the proximal small intestine. BETA2 expression was seen in all enteroendocrine cells and not seen in nonendocrine cells. These results suggest that most small intestinal endocrine cells are developmentally related and that a close developmental relationship exists between secretin-producing S cells and cholecystokinin-producing and L type enteroendocrine cells. In addition, our work shows the existence of a multipotent endocrine-committed cell type and locates this hybrid multipotent cell type to a region of the intestine populated by relatively immature cells.

Key words: Enteroendocrine cells, Secretin, Intestine, Cholecystokinin, Cell ablation, Thymidine kinase

INTRODUCTION

At least 10 different enteroendocrine cell types have been described in the small intestine of mammals (Solcia et al., 1998). Subtype classification relies on the identification of the main hormonal cell content by immunohistochemical methods as well as on the ultrastructure of their secretory large dense core endocrine granules. Several cell types may coexpress more than one hormone. The heterogeneity of hormone expression in enteroendocrine cells is believed to reflect their peculiar multipotency and is observed in gut neuroendocrine tumors and derived cell lines (Klöppel and Heitz, 1988; Madsen et al., 1986; Oie et al., 1983; Pilato et al., 1988; Tischler, 1983). Secretin-producing S cells are located mainly in the small intestine of most mammalian species (Chey and Escoffery, 1976; Larsson et al., 1977; Straus and Yalow, 1978) as well as in the colon (Lopez et al., 1995). In addition, secretin is transiently expressed in insulin-producing β cells of the developing rat endocrine pancreas (Wheeler et al., 1992).

Labeling studies with [3H]thymidine indicate that all four epithelial cell types of the mouse intestine, namely enterocytes, Paneth cells, goblet cells and enteroendocrine cells, originate from a common totipotent stem cell and turnover every 3-4 days (Cheng, 1974; Cheng and Leblond, 1974a,b). In rats, labeling with [3H]thymidine showed that secretin-producing cells turnover once every 4-5 days like the other intestinal cell types (Inokuchi et al., 1986). The absence of labeled secretin cells earlier than 24 hours after the first [3H]thymidine
injection suggested that differentiation towards secretin cells may occur within the crypt in precursor cells expressing secretin at levels below the detection limit of immunohistochemistry.

Coexpression of more than one hormone by individual enteroendocrine cells is consistent with the idea that enteroendocrine cells arise from multipotent endocrine cells. A fraction of intestinal secretin cells coexpress serotonin and substance P, suggesting a developmental relationship between these two lineages (Cetin, 1990). Transgenic mice expressing easily detected reporter genes like human growth hormone (hGH) in intestinal epithelial cells have provided further insight into relationships between different enteroendocrine cell types. The detection of reporter genes such as hGH has proved in some cases to be much more sensitive than directly staining enteroendocrine cells with antibodies directed against specific hormones. Sequences in the first 500 bp of 5' flanking sequence of the liver fatty acid binding protein (L-FABP) gene directed expression of a hGH reporter gene to several enteroendocrine cell types including secretin cells as well as enterocytes, in which L-FABP is normally expressed (Roth and Gordon, 1990; Roth et al., 1990). The results suggested complex lineage differentiation pathways.

We previously showed that 1.6 kb of 5' flanking sequence of the rat secretin gene directed developmentally regulated, cell-specific transgene expression in major gastrointestinal sites of secretin gene expression in transgenic mice. The colocalization of this sensitive reporter gene in multiple enteroendocrine cell types provided the initial evidence that secretin is coexpressed in CCK in the small intestine, and glucagon, peptide-YY and neurotensin cells in the colon. These data were consistent with the existence of a multipotential endocrine-committed cell type from which secretin cells terminally differentiate. However, coexpression of multiple hormones in subpopulations of specific enteroendocrine cell types cannot establish that a particular lineage arises from a common progenitor expressing another hormone.

We have generated transgenic mice expressing the herpes simplex 1 virus thymidine kinase (HSVTK) under control of 1.6 kb of 5' rat secretin gene. HSVTK expression in transgenic mice is nontoxic. However, the viral enzyme renders tissues sensitive to the nucleoside analog ganciclovir, allowing conditional cell ablation. This strategy has been used with success to target cell types for cell-lineage studies allowing conditional cell ablation. This strategy has been used with success to target cell types for cell-lineage studies. Coexpression of more than one hormone by individual enteroendocrine-committed multipotent progenitor cells; (ii) identify the developmental lineage relationship between secretin cells and the other enteroendocrine cell types and (iii) determine the relationship between the putative enteroendocrine-committed progenitor cells and the nonendocrine cell types of the intestinal mucosa. The data reported here indicate that the secretin cell lineage is most closely related to enteroendocrine cells that express CCK and PYT/glucagon which appear to arise from a secretin-producing, multipotent, endocrine-committed cell type. Secretin cells appear to be less closely related to 4 additional endocrine cell types.

### MATERIALS AND METHODS

#### Construction of transgene

1.2 kb of HSVTK coding sequences (~53 to +1438) attached to about 1.2 kb of Simian Virus polyadenylation signal sequences were obtained after BglII digest of the pGK-TK plasmid (a gift from M. Low, Portland, Oregon) and subcloned in pBluescript KS at the BamHI site (pBlueKS-HSVTK). Not-Xho digest from pBlueKS-TK was subcloned in a pIB136-sec vector containing 1.6 kb of 5' flanking sequences of rat secretin gene cloned in the BamHI-SalI site. Correct fragment orientation was confirmed by DNA sequencing before microinjection. The secretin-TK transgene was separated from plasmid vector sequences by digestion with KpnI-Xhol followed by agarose gel electrophoresis.

#### Production of transgenic mice

Purified transgenic was microinjected into the pronucleus of B6D2F1 x B6D2F1 mouse embryos and transferred into the oviducts of pseudopregnant CD1 mice (Hogan et al., 1994). Transgenic mice were identified by DNA blot hybridization with 32P random primed labeled cDNA probes for HSVTK. Transgenic pedigrees were maintained on a CD1 background.

#### Ablation procedure

Sec-HSVTK mice and controls of about 35 g weight (mean 35.5 ± 5.8 g) were treated with 4.8 mg/day of the nucleotide analog ganciclovir (Citovene, Sintex, Ca, USA) for 5 days (Borrelli et al., 1989). Ablation by ganciclovir was accomplished by continuous infusion with subcutaneously implanted osmotic minipumps loaded with either ganciclovir or saline solution according to the manufacturer's instructions (Alzet, model 2002, Alza). Controls consisted either of transgenic mice treated with saline solution or age-matched nontransgenic CD-1 mice treated with ganciclovir. Some transgenic mice were allowed to recover for 5 weeks after treatment to assess endocrine cell repopulation. At the end of the treatment and recovery period, transgenic mice and controls were killed for histochemical studies.

#### Histology and immunohistochemistry

Three samples of about 2 cm each in length from different parts of the small intestine (duodenum, ileum and terminal ileum) were removed, fixed by immersion in Bouin's solution or 10% formalin for 6-8 hours at room temperature and processed into paraffin wax. Serial sections (3-5 μm) were stained with haematoxylin and eosin for conventional histology and PAS/Alcian blue for identification of mucins. Immunohistochemical tests were performed using the avidin-biotinylated peroxidase complex (ABC, Vector, Burlington, USA) method. The following specific sera were used: anti-HSVTK (W. C. Summers, Yale University, New Haven, CT, USA; rabbit polyclonal, 1:2000), secretin (D. Gossens, Free University of Brussels, Belgium; rabbit polyclonal, 1:15,000). Other primary antibodies have been described previously and include rabbit anti-glucagon (1:3,000), rabbit anti-cholecystokinin (1:8,000), rabbit anti-gastrin (1:2,000), guinea pig anti-peptide-YY (PYY) (1:15,000), rabbit anti-somatostatin (1:3,000), rabbit anti-gastric inhibitory polypeptide (GIP, 1:1,000), 5-HT (1:5000), rabbit anti-substance-P (1: 2,000), rabbit anti-neurotensin (1:5,000) (Lopez et al., 1995; Upchurch et al., 1996). Reverse-face sequential immunoperoxidase staining was performed for HSSTK and secretin immunolocalization. Specificity tests for the immunostaining consisted of absorption of each antiserum with its homologous antigen (10 nmol/ml of diluted antiserum), omission of either the primary or secondary antibody, and use of control tissue with or without the pertinent antigen.

Intestinal tissue from BETA2−/− mice was fixed and stained for β-galactosidase activity, embedded, and sectioned at 4 μm as described previously (Mutoh et al., 1998). Following microwave antigen retrieval, sections were immunostained as described previously with
antibodies against secretin, cholecystokinin, GIP, serotonin, substance-P, somatostatin, glucagon, and peptide YY. Primary antibodies were detected by immunoperoxidase using DAB as a substrate.

DNA fragmentation analysis
Sections of formalin-fixed, paraffin wax-embedded samples of small intestine from ganciclovir-treated and control mice were analyzed for DNA fragmentation with a modified TUNEL method (Gavrieli et al., 1993). In brief, after treatment with protease K, sections were incubated with 3' terminal transferase in the presence of biotin-labeled dUTP at 37°C for 2 hours. DNA labeling was revealed using avidin-peroxidase-biotin complexes as above.

Morphometry and statistical analysis
Only tissue samples correctly oriented along the longitudinal axis were used for quantitative analysis. Immunoreactive cells for 9 gastrointestinal hormones were counted per linear millimeter of mucosal length as previously described (Langhans et al., 1997). Data were collected from three different groups of mice: controls, ganciclovir-treated transgenic mice and ganciclovir-treated transgenic mice after recovery. A total of 6 mice, two for each group, were studied. Six to ten observations (mean 7.7) for each cell type were performed for each mouse in all groups. The length of the mucosa available for counting in each mouse, for each specific hormone antiserum ranged from 13.17 mm to 75.27 mm (mean value 37.27 mm). The mean length of mucosa evaluated was 27.84 mm for controls (range 13.17-38.88 mm, 75 observations), 52.29 mm for treated transgenic mice (range 29.59-75.27 mm, 70 observations) and 31.34 mm for treated transgenic mice after recovery (range 18.03-37.69, 63 observations).

Data were first analyzed for equality of population with the Kruskal-Wallis test (analysis of variance) to elicit difference between the three groups. P<0.05 was considered statistically significant. Parameters statistically different at the previous analysis were then compared by the two sample Wilcoxon rank-sum, Mann-Whitney, test to compare data between two groups of observations. A P value of <0.05 was considered statistically significant after Bonferroni (×3) correction.

RESULTS

Thirteen transgenic lineages were established after transgene microinjection. Two transgenic pedigrees with the highest enzymatic activity were selected for further studies with ganciclovir (nos. 933 and 995). Both pedigrees showed a similar phenotype following treatment and one (no. 995) was used for subsequent detailed studies. Transgenic mice were normal in size and reproduced normally. To assess the correct tissue-specific expression of the transgene, samples of the small intestine of adult transgenic mice were investigated by immunohistochemistry with an antisera specific for HSVTK. HSVTK immunoreactivity was detected in discrete mucosal cells of the small intestine (Fig. 1A) which coexpressed secretin in the adjacent section (Fig. 1B), thus confirming that transgene expression was specifically directed to secretin cells.

The small intestine from ganciclovir-treated transgenic mice showed relatively normal crypt-villus cytoarchitecture indicating that most cells in the intestine were spared (Fig. 2A). Paneth cells, enterocytes and goblet cells appeared relatively normal (not shown). Thus the drug did not induce relatively nonspecific, generalized ablation that could potentially occur due to reuptake of phosphorylated ganciclovir released from correctly targeted dying cells. Examination at higher magnification revealed small numbers of cells in the mid to lower regions of the crypt compartment with pyknotic, hyperchromatic nuclei and shrunken cytoplasm, features typical of cells undergoing apoptosis (Fig. 2B, arrows). In contrast, we failed to identify apoptotic cells in the crypts of control animals. Some of the dying cell stained for secretin immunoreactivity (Fig. 3B).

Staining for DNA fragmentation by the TUNEL method in samples of the small intestine of ganciclovir-treated transgenic mice confirmed the histologic observations (Fig. 2B). In ganciclovir-treated transgenic mice, numerous cells showed nuclear staining for DNA fragmentation, indicative of apoptosis, in the lower to mid portion of the crypts (Fig. 2C,E). Apoptotic cells were not seen in the crypts of either saline-treated transgenic mice or in ganciclovir-treated CD1 mice. In addition, relatively rare cells with labeled nuclei were observed at the tip of the villus in both transgenic and control mice (Fig. 2C,D). The occasional dying cells at the villus tip probably represent apoptosis that occurs during normal epithelial cell turnover in the intestine. These features indicate that the cell death in the neck zone of the crypt of ganciclovir-treated transgenic mice was a specific effect of transgene expression and ganciclovir treatment.

We examined the small intestine from control mice and ganciclovir-treated transgenic mice with a panel of ten specific antisera directed against secretin and nine gastrointestinal hormones. In ganciclovir-treated transgenic mice, secretin immunoreactive cells were reduced in number compared to controls (Fig. 3A,B). A significant fraction of the few remaining secretin cells appeared abnormal with pyknotic nuclei with cytoplasmic shrinkage (Fig. 3D) when compared to secretin cells in control animals (Fig. 3C), suggesting that they were undergoing programmed cell death. The numbers of most other enteroendocrine cells appeared to be reduced in addition.

A detailed morphometric analysis for 9 intestinal endocrine cell types enabled us to quantitate the effect of ganciclovir treatment on different enteroendocrine populations of transgenic mice (Fig. 4). The mean number of secretin cells was reduced by 86% in transgenic mice as compared to control transgenic animals treated with saline (Fig. 4). Unexpectedly, cells expressing CCK (89% reduction), glucagon (85%) and peptide YY (88%) were similarly reduced in number in ganciclovir-treated transgenic mice, suggesting a close developmental relationship between these cell lineages.

Several populations were partially depleted by ganciclovir treatment, including GIP cells (59%), substance-P (58%), somatostatin (55%) and 5HT (45%) cells. In contrast, gastrin-immunoreactive cells decreased only by 13% suggesting that they were not significantly affected by ganciclovir treatment. The number of endocrine cells of transgenic mice returned to values similar to controls after 5 weeks recovery, indicating that the stem cell population was spared (Fig. 4). The extremely low number of neurotensin-expressing cells in the small intestine precluded meaningful quantitative analysis of this lineage. The equality of population test (variance analysis) revealed that observed differences between ganciclovir-treated transgenic mice and either controls or transgenic animals postrecovery were statistically significant (P≤0.006) for all enteroendocrine cell types examined except gastrin-immunoreactive (G) cells P=0.08. Comparison of the groups...
other than gastrin cells (Mann-Whitney test) showed that results from treated transgenic mice were statistically different when compared to those of controls or transgenic mice after recovery (observed $P \leq 0.03$). Conversely, no statistically significant difference was observed between controls and transgenic mice after recovery.

We previously showed that mice containing a targeted deletion in the gene encoding the basic helix-loop-helix transcription factor, BETA2, failed to develop both secretin- and CCK-expressing enteroendocrine cells, leading us to suggest a close relationship between these two lineages (Naya et al., 1997). To determine whether expression of BETA2 was related to the observed results in cell lineage ablation experiments, we examined small intestine endocrine cell types for expression of BETA2 using mice that we have previously described, containing a targeted deletion in a single BETA2 allele. Most of the region coding for BETA2 was replaced with an in frame fusion of the $\textit{lacZ}$ reporter gene, allowing us to identify cells expressing $\beta$-galactosidase activity by X-gal histochemistry (Naya et al., 1997). The sensitivity of this method is far greater than immunostaining with BETA2 antibodies described earlier (Mutoh et al., 1997). Nuclear $\beta$-galactosidase activity was seen in each of the small intestinal endocrine cell types examined in the proximal small intestine (Fig. 5). $\beta$-galactosidase staining was less intense in the distal small intestine where most peptide YY/glucagon cells are found. However, the majority of the peptide YY/glucagon cells showed $\beta$-galactosidase activity. To determine if BETA2 is expressed only in enteroendocrine cells, we stained sections of recovery (observed $P \leq 0.03$). Conversely, no statistically significant difference was observed between controls and transgenic mice after recovery.

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small intestine for both β-galactosidase activity and chromogranin A, a marker for endocrine secretory granules. All chromogranin A immunoreactive cells showed staining for β-galactosidase activity and virtually all X-gal-stained cells showed colocalized staining for chromogranin A, indicating that most if not all small intestinal enteroendocrine cells express BETA2 and that BETA2 expression is restricted to endocrine cells in the intestine.

**DISCUSSION**

This paper describes the effect of inducible selective ablation of secretin cells in the small intestine of transgenic mice. The 1.6 kb of 5′ sequence used to drive expression of herpes virus 1 thymidine kinase (HSVTK) is sufficient to direct developmentally regulated tissue and cell-specific expression of reporter genes in transgenic mice (Lopez et al., 1995). The sensitivity of secretin-producing enteroendocrine cells to ablation after 5 days treatment with ganciclovir indicates that the secretin gene is expressed in proliferating cells. Prior to the present work, it was believed that expression of secretin was restricted to terminally differentiated, non-proliferating cells based on the localization of secretin cells exclusively to the nondividing villus compartment of the small intestine as opposed to the proliferating crypt compartment. The failure of secretin cells to incorporate radiolabel from a single injection of [3H]thymidine into rats provided additional evidence that secretin cells were nonreplicating.

Continuous [3H]thymidine labeling showed increased incorporation of the isotope into secretin cells with all cells labeled after 5 days, the renewal time for small intestinal enteroendocrine cells. Thus secretin cells were believed to arise from a less differentiated, continuously self-renewing progenitor cell. The present work supports the existence of a proliferating progenitor cell and indicates that a subpopulation of cells destined to differentiate into secretin cells continue to divide and express the secretin gene. This subpopulation of cells appears to express secretin at levels below the limits of detection by immunohistochemistry.

Histology and DNA fragmentation analyses indicate that the cells targeted by the nucleoside analog in transgenic mice are located in the mid portion of the crypt in contrast to mature secretin cells found mainly in the villus compartment. The totipotent intestinal stem cell as well as other relatively immature cells are believed to reside in this part of the intestinal gland. Therefore, the ablated secretin-expressing cells in Sec-HSVTK mice appear to be located physically close to relatively undifferentiated cells, suggesting that cells were targeted at an early stage of differentiation, after committed

**Fig. 5.** BETA2 is expressed in multiple enteroendocrine lineages in the small intestine. Sections from a 1-month old BETA2+/− mouse showing nuclear β-galactosidase activity (blue) colocalized with (large arrows) chromogranin A (A), cholecystokinin (B), serotonin (C), GIP (D), somatostatin (E), and peptide YY (F). Small arrows in B indicate cells expressing β-galactosidase activity that do not stain for cholecystokinin. Hormones stained by immunoperoxidase, hematoxylin counterstain; scale bar, 20 μm (A-C); 10 μm (D-F).
enteroendocrine progenitor cells segregate from nonendocrine lineages and stem cells. This population of immature cells expresses relatively low levels of secretin and thymidine kinase yet is ablated by ganciclovir due to the sensitivity of cells to the drug. Continuous infusion of ganciclovir for 5 days, the approximate turnover time for secretin cells, resulted in depletion of greater than 90% of secretin cells. Thus, it is likely that the nucleoside is not directly targeting mature villus secretin cells but rather, an immature, proliferating progenitor population.

A small fraction of secretin cells (about 10%) survived after 5 days of ganciclovir treatment. These cells may represent a small subpopulation of terminally differentiated secretin cells that turnover more slowly. It is also possible that these cells were targeted by the nucleoside but are only just entering the apoptotic pathway and have yet to show morphologic changes consistent with programmed cell death. Although unlikely, we cannot exclude the possibility that the transgene may not be expressed in the few secretin cells surviving ablation.

It is well established that enteroendocrine cells frequently express more than one hormone. Coexpression of serotonin (5HT) and substance-P in a fraction of secretin cells has been well documented (Cetin, 1990; Roth and Gordon, 1990; Roth et al., 1990). Subpopulations of small intestinal CCK-expressing (Lopez et al., 1995) and glucagon- (GLP-1) expressing cells (Aiken et al., 1994) coexpress secretin, further suggesting that S type enterroendoendocrine cells are related to several other enterondocrine lineages. Ganciclovir treatment of SEC-HSVTK mice resulted in depletion of CCK-expressing and PYY/glucagon-expressing (type L cells) enteroendocrine cells in addition to secretin cells. This finding suggests a much closer lineage relationship between secretin cells, CCK and PYY/glucagon cells than was previously appreciated from coexpression studies. The data presented here confirms that at least CCK and PYY/glucagon cells share a common differentiation pathway with secretin cells until almost terminally differentiated.

Ganciclovir treatment of Sec-HSVTK transgenic mice depleted cell lineages producing GIP, substance-P, serotonin and somatostatin to a lesser extent than cells producing secretin, CCK and PYY/glucagon. This finding may suggest that such cell types are also related to secretin cells in their lineage differentiation pathway. Alternatively, selected subpopulations of these four cell types may be able to express secretin before terminal differentiation. Although unlikely, we cannot exclude that loss of secretin after ablation may reduce expression of other hormones leading to an apparent depletion of its cell type. In contrast to the other small intestinal endocrine cell lineages, gastrin-producing cells were unaffected by treatment with ganciclovir, indicating a separate differentiation pathway with respect to secretin cells. After cessation of ganciclovir treatment, each of the depleted cell lineages repopulates the intestine to pretreatment numbers. The ability of each enteroendocrine lineage to recover suggests that each lineage arises from an earlier progenitor/stem cell that does not express the secretin gene and is therefore not targeted by ganciclovir.

We previously showed that the same region of the secretin gene 5′ flanking sequence directed the expression of a human growth hormone reporter gene to secretin cells as well as subpopulations of CCK, substance-P, and serotonin cells in the small intestine of transgenic mice. However, the transgene was not expressed in small intestine endocrine cells expressing glucagon, peptide YY, somatostatin, GIP or gastrin. The ablation of a significant fraction of glucagon/peptide YY, somatostatin and GIP cells reported here was not anticipated in light of our earlier observations that the secretin gene or a secretin promoter driven reporter did not appear to be coexpressed in these cells (Lopez et al., 1995). Secretin expression may fall below the limits of detection by immunostaining in the latter 3 cell lineages. Only 10% of CCK cells coexpress secretin, yet treatment with ganciclovir killed over 90% of this cell type. We interpret these results to indicate that relatively low levels of thymidine kinase expression driven by the secretin gene are sufficient to confer sensitivity to ganciclovir. The results suggest that cell lineage ablation is a much more sensitive approach to study enteroendocrine cell lineage than hormone coexpression. We have previously shown that the same 1.6 kb of secretin gene 5′ flanking sequence directs tissue specific, cell-specific and developmentally regulated expression of reporter genes in transgenic mice, making it unlikely that the observed ablation of glucagon/peptide YY, somatostatin and GIP cells resulted from promiscuous expression of thymidine kinase (Lopez et al., 1995).

Transgenic mouse studies examining expression of human growth hormone (hGH) under control of the L-FABP gene in enteroendocrine cells showed hGH in small intestinal endocrine cells expressing secretin, CCK, GIP, serotonin, substance P, and glucagon (GLP-1), suggesting that each of these cell lineages may share a common developmental program. In the present work we show expression of the basic helix-loop-helix transcription factor, BETA2, also known as neuro D, in cell types targeted by ganciclovir. The expression of BETA2 in all small intestine enteroendocrine cells provides further evidence that multiple enteroendocrine cell lineages are developmentally related.

The close relationship between secretin and CCK-expressing enteronerdocrine cells is further highlighted by the failure of mice containing a targeted deletion of BETA2 to develop either secretin or CCK cells (Naya et al., 1997). This protein has a major role in the terminal differentiation of secretin-expressing enteroendocrine cells by coordinating transcription of the secretin gene with cell cycle arrest and cell turnover (Mutoh et al., 1998). The requirement for a functional BETA2 protein for the development of secretin and CCK cells is an example of a common differentiation pathway shared by these two cell lineages. We previously noted the presence of other enteroendocrine cell lineages in BETA2−/− mice in contrast to secretin and CCK cells. Thus, this protein may be a marker of enteroendocrine cells, but is not required for transcription of genes that lead to expression of GIP, substance-P, glucagon, peptide YY, serotonin and somatostatin.

In summary, enteroendocrine cells appear to differentiate from progenitor cells expressing more than one hormone through a multistep process (Fig. 6). Cell lineage ablation experiments imply the existence of at least two types of multipotent enteroendocrine progenitor cells. Secretin-expressing enteroendocrine cells differentiate from an endocrine-committed progenitor cell type that may give rise to other cell lineages. This earliest progenitor does not express the secretin gene and in our transgenmic mice was not sensitive
The totipotent stem cell generates an early endocrine-committed multipotent cell type that does not express the secretin gene. This early progenitor cell can differentiate into a secretin-expressing progenitor that is sensitive to ablation with ganciclovir in secretin-HSVTK mice. Most if not all secretin-, secretin-expressing progenitor that is sensitive to ablation experiments also indicate that some enteroendocrine cells do not arise from the secretin-expressing progenitor as does a significant fraction (approximately 50%) of SP, 5HT, SOM and GIP cells. The secretin-expressing progenitor is not an obligatory intermediate for the differentiation of gastrin-expressing cells (Gas), as well as a significant fraction (approximately 50%) of substance-P (SP), serotonin (5HT), somatostatin (SOM) and GIP cells, hence they are not targeted by ganciclovir in secretin-HSVTK mice.

FIG. 6. Model for enteroendocrine cell differentiation in the small intestine of mouse. The totipotent stem cell generates an early endocrine-committed multipotent cell type that does not express the secretin gene. This early progenitor cell can differentiate into a secretin-expressing progenitor that is sensitive to ablation with ganciclovir in secretin-HSVTK mice. Most if not all secretin-, secretin-expressing progenitor that is sensitive to ablation experiments also indicate that some enteroendocrine cells do not arise from the secretin-expressing progenitor as does a significant fraction (approximately 50%) of SP, 5HT, SOM and GIP cells. The secretin-expressing progenitor is not an obligatory intermediate for the differentiation of gastrin-expressing cells (Gas), as well as a significant fraction (approximately 50%) of substance-P (SP), serotonin (5HT), somatostatin (SOM) and GIP cells, hence they are not targeted by ganciclovir in secretin-HSVTK mice.

to ganciclovir. A fraction of the earliest progenitor cells subsequently differentiate into late intermediate cells expressing the secretin gene. At least three terminally differentiated enteroendocrine lineages including secretin cells, CCK cells and glucagon peptide YY cells segregate from these late progenitor cells as an obligate intermediate step. Cell lineage ablation experiments also indicate that some enteroendocrine cells do not arise from the secretin-expressing intermediate cells as is likely for gastrin-expressing cells. Other cell lineages expressing GIP, substance-P, somatostatin and serotonin may terminally differentiate through two distinct pathways. Approximately half of enteroendocrine cells expressing substance-P, 5HT, GIP and somatostatin arise from the secretin-expressing progenitor cell type whereas the other half differentiate from an earlier committed entodocrine cell progenitor without ever expressing the secretin gene.

Our data reveal a much closer developmental relationship between different enteroendocrine cell types than was previously appreciated from hormone coexpression studies, attesting to the sensitivity of cell lineage ablation as a method for studying cell lineage relationships. This work raises several interesting questions about how intestinal endocrine cells differentiate. First, does each cell type differentiate from the proliferating multipotent progenitor on the basis of a predetermined differentiation cascade or (and) in response to specific physiological stimuli? Secondly, since a number of enteroendocrine cell types share a significant part of their differentiation pathways, can the phenotype change so that one cell type may differentiate into another?

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