Further Studies Support the Participation of Stem Cells in the Cell Turnover of Duodenal Adenomas

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Abstract. *Backgroun: In the normal duodenal mucosa, differentiated cells (enterocytes, goblet cells and endocrine cells) migrate from stem cells to the tip of the villi, but the lysozyme-producing Paneth cells migrate to the bottom of the crypts. The position of the Paneth cells within duodenal adenomas was investigated. Patients and Methods: Sections from 83 duodenal adenomas were stained with hematoxylin-eosin (H&E) and with anti-lysozyme. Mature Paneth cells were those showing coarse brightly red cytoplasmic granules in H&E stain whereas their precursors were the lysozyme-positive cells that were undetected by H&E. Results: The number of mature Paneth cells/high power field (x 40) varied in adenomas from 4 to 12 (mean 6.5) in H&E stain, while 32 to 62 cells/field (mean 46.5) were positive in anti-lysozyme immunostain ($p<0.05$). The lysozyme-expressing cells were randomly distributed within the adenoma including the superficial cell layers. Discussion and conclusion: Since mature Paneth cells and their precursors are positioned underneath stem cells, the presence of mature Paneth cells and their lysozyme-positive precursors in the surface epithelium of duodenal adenomas would imply that stem cells might have already exfoliated. An alternative explanation would mean that mutated stem cells, anchored in the bottom of the crypts of the adenoma would redirect, in an unparalleled fashion, the ontogenetic logistics of migration for Paneth cells. This stochastic molecular behaviour would require a reversal from the pre-determined migratory flow for Paneth cells to a paradoxical migration mode for these cells (from stem cells vertically along the villus, before exfoliation). Consequently, it is not inconceivable that stem cells might participate, together with other mature cells, in the cellular turnover of duodenal adenomas. If that is the case, the duodenal adenoma emerges as a suitable model to monitor the actual fate of mutated stem cells.*

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Cell renewal systems require synchronized instructions to preserve equilibrium between cell proliferation and differentiation (1-3). Stem cells are the only cells in a tissue that persist long enough to undergo the long-lasting sequence of mutations and selection required for multistage carcinogenesis (4). In the duodenal mucosa, pericryptal myofibroblasts produce the wingless gene (*Wnt*) signalling ligands, that access the Frizzled receptors on the basal epithelial stem cells of the crypts. Factors such as bone morphogenetic protein (BMP), its antagonists gremlin 1 (GREM1) and gremlin 2 (GREM2), Notch signals and the Wnt effect on ephrin B1 (EPNB1) and its receptors EPNB2/EPNB3, control the behaviour and maintenance of stem cells and cell migration and differentiation (5-11).

The duodenal mucosa is histologically organized into crypts and villi. The stem cells are confined to the basal aspect of the crypts, from where they are capable of self-renewal, generating several types of more committed precursor cells through life (4). These committed precursor cells actively divide within the proliferative compartment. Following several divisions, the precursors generate differentiated mature cell families of enterocytes (absorptive cells) and secreting cells (goblet cells, enteroendocrine cells and Paneth cells). One of these cell types migrates from the stem cells in a retrograde fashion: the Paneth cells migrate downwards towards the base of the crypt, where they normally reside for about 20 days before being engulfed by phagocytes (1, 2). In contrast, the secreting cells migrate upward, along the villus vertical axis before exfoliation (4). Paneth cells also differ from the other three cell phenotypes, in as much as they secrete lysozyme and α-defensins (12-19), termed cryptdin, key peptides that keep the duodenum and the rest of the small intestine free from pathogenic bacteria.

In previous studies on duodenal adenomas (20-22), it became clear that Paneth cells could be present at all epithelial levels within the histological profile of the adenomas.

In the present investigation, the position of the Paneth cells within the histological profile of duodenal adenomas was studied in a substantial number of cases. The aim was to understand the logistics of the stem cells in these neoplasias, considering the unique position of the Paneth cells in the crypts, namely underneath the stem cells.

Patients and Methods

Eighty-three, endoscopically removed duodenal adenomas were investigated. Sixty-four adenomas were from familial adenomatous polyposis (FAP) patients and the remaining 19 from patients who had no clinical history of FAP (sporadic adenomas). Normal duodenal mucosa was also present in 40 out of the 83 adenomas.

Sections were stained with hematoxylin-eosin (H&E) and with anti-lysozyme immunostain (Lysozyme, DAKO, Glostrup, Denmark). In addition, sections of duodenal mucosa from 30 patients that were histologically normal in H&E stain were challenged with anti-lysozyme immunostain.

Mature Paneth cells were regarded as cells showing coarse brightly red cytoplasmic granules in H&E stain and Paneth cell precursors were considered to be those lysozyme-expressing cells that remained undetected in H&E stain (that is without coarse brightly red cytoplasmic granules).

The presence of mature Paneth cells (H&E) and their precursors (lysozyme immunostain) found within the confines of the lower or of the upper halves of the crypts in the adenomas were registered separately, at ×4 magnification.

The number of Paneth cells in H&E stain and in lysozyme immunostain was assessed, in the most populated fields, at ×40 magnification.

Statistical analysis. The non-parametric Wilcoxon test was carried out using StatView Version 4.5 software (Abacus Concepts, Berkley, CA, USA). Differences were considered significant at 95% confidence level ($p<0.05$).

Results

Mature Paneth cells (H&E stain) in the normal duodenal mucosa. Mature Paneth cells were found at the bottom of the duodenal crypts in all 40 biopsies of normal tissues from the FAP patients harbouring a duodenal adenoma and in the 30 duodenal biopsies obtained from normal individuals (Figure 1). Mature Paneth cells were not found at any other level in the normal duodenal mucosa. The number of mature Paneth cells/high power field varied from 3 to 6 (mean 3.5) in the bottom of the crypts in the normal duodenal mucosa from the FAP and non-FAP patients harbouring a duodenal adenoma and in the normal individuals.

Mature Paneth cells (H&E stain) in duodenal adenomas. Mature Paneth cells were recorded in the lower half of the crypts in 84.3% (70/83) of the adenomas and in the upper half in 75.9% (63/83) (Figure 2). All the adenomas having mature Paneth cells in the upper half also had mature Paneth cells in the lower half. Seven adenomas had mature Paneth cells only in the lower half of the lesion.

The number of mature Paneth cells/high power field ranged in the duodenal adenomas from 4 to 12 (mean 6.5). No essential difference was found between FAP and sporadic adenomas.

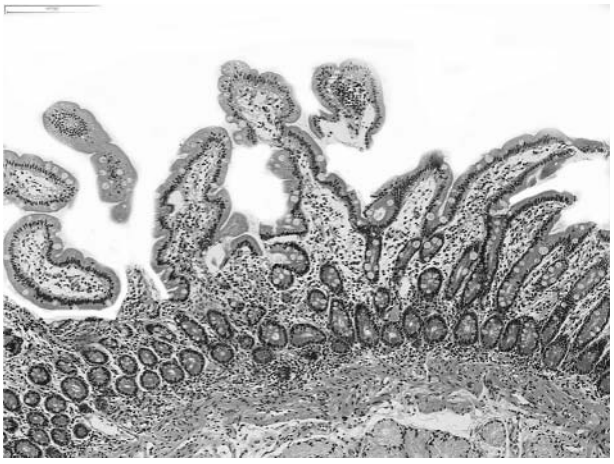

Figure 1. *Normal duodenal mucosa. Note Paneth cells at the bottom of the crypts (H&E, original magnification ×4).*

Mature Paneth cells and their precursors (anti-lysozyme immunostain) in the normal duodenal mucosa. Lysozyme-expressing cells were found in the bottom of the duodenal crypts in all 40 biopsies of normal tissue from the FAP patients harbouring a duodenal adenoma and in all 30 duodenal biopsies obtained from normal individuals (Figure 3). Lysozyme-expressing cells were not found at any other level in the normal duodenal mucosa.

The number of lysozyme-expressing cells/high power field in the cypts from the normal duodenal mucosa, both from the FAP patients harbouring a duodenal adenoma and from the normal individuals, as previously reported (17), was difficult to assess. This was due to the overlapping of the immunological reaction in the Paneth cells, normally located at the bottom of the crypts.

Mature Paneth cells and their precursors (anti-lysozyme immunostain) in duodenal adenomas. Lysozyme-expressing cells were found both in the lower half and in the upper half of the crypts in all (100%) of the 83 adenomas (Figure 4). The number of lysozyme-expressing cells/high power field ranged from 32 to 62 (mean 46.5).

The difference between the presence of mature Paneth cells in H&E stain and of lysozyme-expressing cells in the 83 adenomas was significant ($p<0.05$), as well as the difference between the number of mature Paneth cells/high power field (H&E stain) and of lysozyme-expressing cells/high power field in the 83 adenomas ($p<0.05$). No essential difference was found between the FAP and sporadic adenomas.

Discussion

Studies on mice have indicated that development and differentiation in the small intestine are under the control of at least three signalling pathways known as canonical Wnt,

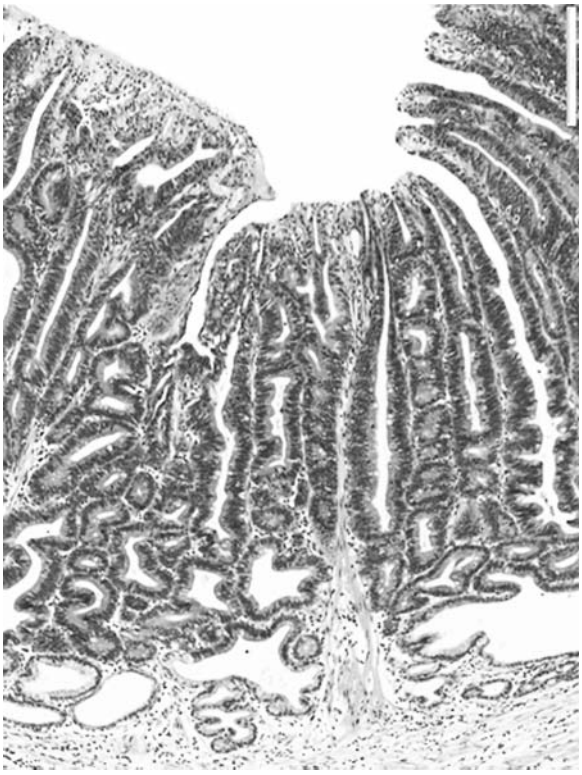

Figure 2. *Duodenal adenoma. The few mature Paneth cells present are difficult to discern (H&E, original magnification ×10).*

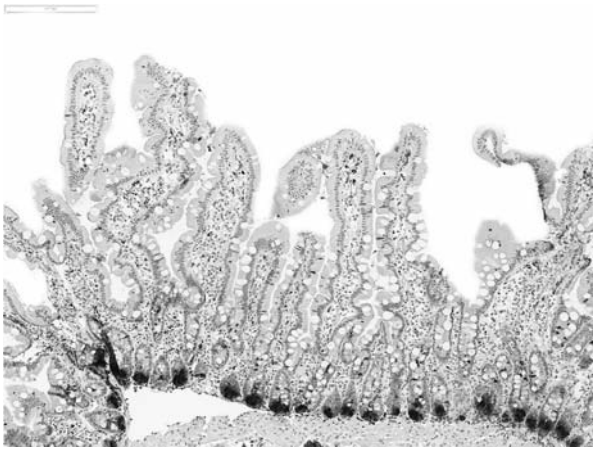

Figure 3. *Normal duodenal mucosa. Note lysozyme-expressing, dark, easily detected Paneth cells at the bottom of the crypts (lysozyme immunostain, original magnification ×4).*

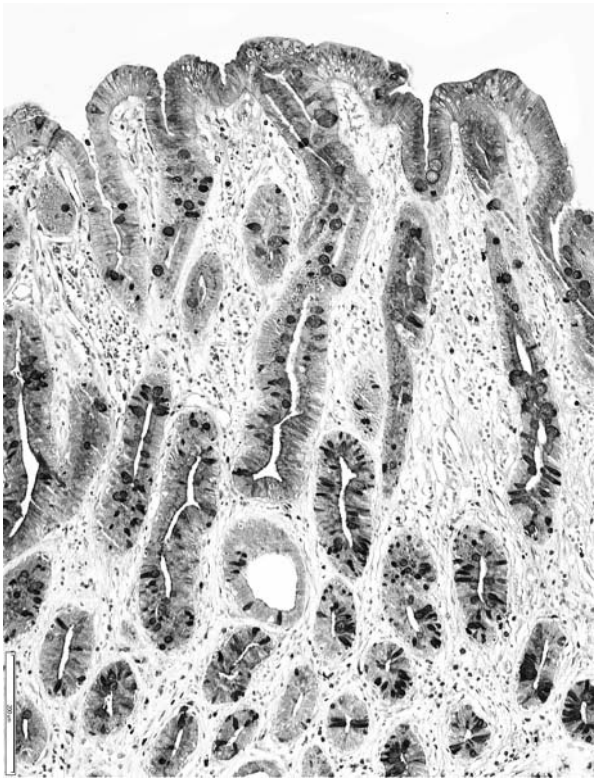

Figure 4. *The same duodenal adenoma as shown in Figure 2. Note numerous lysozyme-expressing cells (mature and precursors), some of them in the superficial epithelial layer of the adenoma (lysozyme immunostain, original magnification ×10).*

Notch and hedgehog (2, 22-27). The canonical Wnt pathway is the key regulator of proliferation of the stem cells in the crypts, its activation being essential for the maintenance of the crypt cell population in a proliferate state (27, 28). Varnat *et al.* (29) recently showed that peroxisome proliferator receptor β (PPARβ) a nuclear hormone receptor in the gastrointestinal tract, is expressed at the bottom of the crypts where Paneth cells reside. PPARβ partly controls Paneth cell homeostasis by down-regulating the expression of the Indian hedgehog (ihh), a signal sent by mature Paneth cells to differentiate their precursors (29). The trans-amplifying progenitors are under the Wnt signal domain, whereas differentiated cells, other than Paneth cells, are present in areas where the canonical Wnt signal is inactive (27, 28). A family of bHLH (basic-helix-loop-helix) transcription factors, and its upstream Notch signalling, plays a fundamental role in regulating the differentiation and cell type specification of intestinal epithelial cells (27, 28). Cdc42 (a small GTPase activated by integrins, growth factor, cytokine receptors and cadherins) is important for the terminal differentiation of progenitor cells, regulating β-catenin degradation (30). In the small intestine the nuclear accumulation of β-catenin is mainly located in cells at the base of the crypts and decreases as the cell moves toward the top of the crypts (25). Thus, β-catenin accumulation correlates with areas of cell proliferation, whereas β-catenin degradation occurs in areas in which cell differentiation occurs. Hence, several molecular signals participate in the regulation of progenitor cells, of mature Paneth cells and their precursors.

Lysozyme-expressing cells were found haphazardly distributed within the histological profile of the lesion, including the most superficial cell layers of the adenomatous glands in the present study. The latter finding suggested that if the innate code that choreographs the direction of cell migration for Paneth cells was also valid for duodenal adenomas, the stem cells normally overlying the Paneth cells would have exfoliated into the lumen of the organ. A less likely alternative explanation would be that mutated stem cells, anchored in the bottom of the adenomatous crypts would redirect, in an unparalleled fashion, the ontogenic logistics of migration of the Paneth cells. This stochastic molecular decision would imply a reversal of the migration signals for Paneth cells to a highly aberrant, paradoxical migration flow, from stem cells towards the villus vertical axis, before exfoliation. Such corrupt cell migration for the Paneth cells would defy the innate by-laws of cell migration from stem cells in the duodenal mucosa.

In the light of these considerations, it is not inconceivable that stem cells, together with the other differentiated cells (dysplastic enterocytes, Paneth, goblet and endocrine cells), participate in the cellular turnover of duodenal adenomas. If that is the case, the duodenal adenoma emerges as a suitable model to monitor the fate of mutated stem cells.

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