Int J Clin Exp Pathol (2009) 2, 149-153
www.ijcep.com/IJCEP805004

Original Article

Stem Cells might Participate in the Cell Turnover of Duodenal Adenomas

Carlos A Rubio

Gastrointestinal and Liver Pathology Research Laboratory, Department of Pathology, Karolinska Institute and University Hospital, 17176, Stockholm, Sweden

Received 17 May 2008; Accepted in revision 30 June 2008; Available online 10 July 2008

Abstract: Eighty-three duodenal adenomas were stained with hematoxylin-eosin (H&E) and with anti-lysozyme immunostain. Mature Paneth cells were those showing coarse brightly red cytoplasmic granules in H&E stain and Paneth cell precursors were those lysozyme-expressing cells that had remained undetected in H&E stain. In the adenomas, the number of mature Paneth cells/high power field varied from 4 to 12 (mean 6.5) in H&E stain, and of lysozyme-expressing cells from 32 to 62 (mean 46.5) ($p<0.05$). Lysozyme-expressing cells were found haphazardly distributed within the histological profile of the lesion, even in the most superficial cell layer of the dysplastic glands. The latter suggests that if the innate programmed vector of cell migration were valid for duodenal adenomas (from stem cells towards the bottom of the crypts for Paneth cells) the stem cells, normally overlaying Paneth cells, would have been exfoliated into the lumen. Another, less likely option, is that mutated stem cells at the bottom of duodenal adenomas translocate, in an unprecedented manner, the ontogenic signals of migration for Paneth cells. This stochastic molecular option would imply a total reversal of the normal migratory vector for Paneth cells, to a more aberrant, paradoxical migration flow, from stem cells to the villus domain, before exfoliation. Because of the unique migratory direction of the Paneth cells in the crypts of Lieberkühn, the duodenal adenoma emerges as a suitable histo-biological model to monitor the fate of stem cells. It is suggested that stem cells, together with the other recordable mature cells, namely dysplastic enterocytes, Paneth cells and goblet cells, participate in the cell turnover of duodenal adenomas. Further studies are necessary to definitively validate the abovementioned suggested hypothesis.
Key Words: Duodenum, adenomas, Paneth cells, stem cells, migration, turnover

Introduction

The cell renewal machinery in the duodenal mucosa requires coordinated regulation to maintain balance between proliferation and differentiation. According to Nakamura *et al* [1] the stem cells, spatially confined to the lower part of the crypt, are able to self-renew through life generating several types of more committed precursor cells that actively divide within the proliferative compartment. After several divisions, these precursors differentiate into four mature cell lineages: absorptive enterocytes and three secreting cells, namely goblet, enteroendocrine and Paneth cells [2]. The migratory direction of these differentiated cells along the mucosa differs from each other. Paneth cells migrate downwards towards the base of the crypt where they normally reside for about 20 days before being engulfed by phagocytes. Enterocytes, goblet and enteroendocrine cells, on the other hand, migrate upward along the villus vertical axis before exfoliation [3].

Paneth cells secrete lysozyme and α-defensins [4-9], key peptides that keep the duodenum and the rest of the small intestine free from pathogenic bacteria. In previous studies we assessed the frequency of duodenal adenomas having gastric-duodenal metaplasia [10] and reported the first case of serrated adenoma of the duodenum [11]. From these studies it became apparent that Paneth cells could be found at various levels within the adenomas. Preliminary results on the distribution of Paneth cells in tissue sections of 6 duodenal adenomas were recently reported [12]. In the present communication, the spatial position of Paneth cells was further

investigated in 83 duodenal adenomas. The aim was to explore whether the spatial position of the Paneth cells within the adenoma could be of help in deducing the possible final fate of stem cells in these neoplasias.

Material and Methods

Tissue Samples

A total of 83 endoscopically-removed duodenal adenomas were investigated. 64 were from patients with familial adenomatous polyposis (FAP) and the remaining 19 from patients without a clinical history of FAP, so-called sporadic adenomas.

Sections from the 83 duodenal adenomas were stained with hematoxylin-eosin (H&E) and with anti-lysozyme immunostain (Lysozyme, DAKO, Glinstrup, Denmark). In 40 of the 83 adenomas, the biopsies also contained histologically normal duodenal mucosa. In addition, 30 biopsies from normal subjects showing histologically normal duodenal mucosa were also stained with H&E and with anti-lysozyme immunostain.

Definition

Mature Paneth cells were those showing coarse brightly red cytoplasmic granules in H&E stained sections. Precursors of Paneth cells were those expressing lysozyme without the bright red coarse granules seen in H&E stained sections.

Topographical Localization of Mature and Precursor Paneth Cells

Mature Paneth cells in the H&E stain and precursor Paneth cells in the lysozyme immunostain found within the confines of the lower or upper portion of the adenomas were registered separately, using 4x magnifications. The topographical position of the mature Paneth cells (H&E) and lysozyme-expressing Paneth cell precursors in the histologically-normal duodenal mucosa was also recorded.

Semi-quantification of Mature and Precursor Paneth Cells

The number of mature Paneth cells (H&E) and cells expressing lysozyme was assessed in the most populated field using 40x magnifications.

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

Topographic Localization of Mature Paneth Cells in H&E Stain

In the normal duodenal mucosa, mature Paneth cells were found at the bottom of the crypts of Lieberkühn in all 40 biopsies from FAP patients harbouring a duodenal adenoma and in the 30 duodenal biopsies obtained in normal subjects. They were not found at other levels in the epithelium (**Figure 1**).

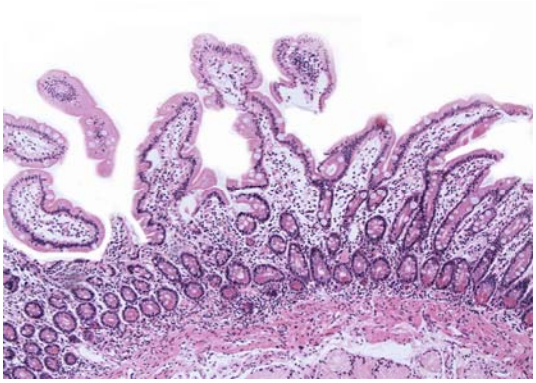


Figure 1 Normal duodenal mucosa with Paneth cells at the bottom of the crypts (H&E, original magnification 4x).

In duodenal adenomas, mature Paneth cells were observed in the lower half in 84.3% (70/83) of the adenomas and in the upper half in 75.9% (63/83). The 63 adenomas having mature Paneth cells in the upper half also showed mature Paneth cells in the lower half (**Figure 2**). Thus, only 7 adenomas had mature Paneth cells in the lower half of the lesion.

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 normal subjects as well as from patients with FAP and non-FAP harbouring a duodenal adenoma. In duodenal adenomas, the number of mature Paneth cells/high power field varied from 4 to 12



Figure 2 Duodenal adenoma with a few mature Paneth cells (H&E, original magnification 10x).

(mean 6.5). No significant difference was found between FAP and sporadic adenomas.

Topographic Localization of Precursor Paneth Cells in Lysozyme Immunostain

In the normal duodenal mucosa, lysozyme-expressing cells were found in the bottom of the crypts of Lieberkühn in all 40 biopsies from FAP patients harbouring a duodenal adenoma and in all 30 duodenal biopsies obtained in normal subjects (**Figure 3**). Lysozyme-expressing cells were not found at other levels in the normal duodenal epithelium.

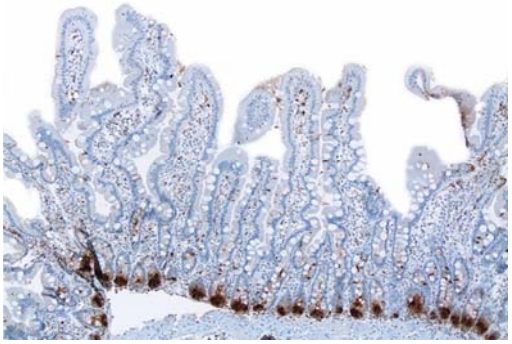


Figure 3 Normal duodenal mucosa with lysozyme-expressing, dark, easily detected Paneth cells at the bottom of the crypts (lysozyme immunostain, original magnification 4x).

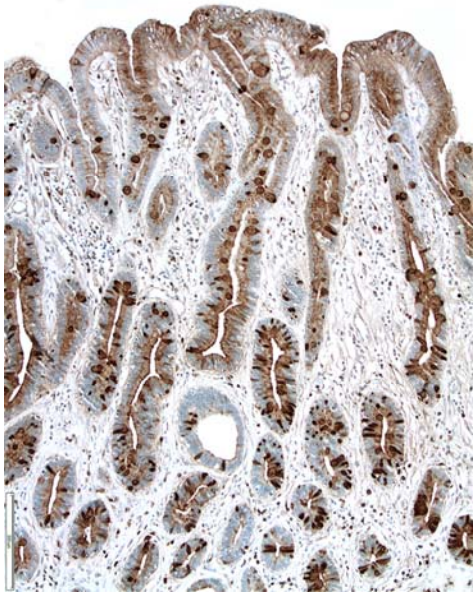


Figure 4 Duodenal adenoma with numerous lysozyme-expressing cells (mature and precursors), some of them in the superficial epithelial layer of the adenoma (lysozyme immunostain, original magnification 10x).

In duodenal adenomas, lysozyme-expressing cells were recorded both in the lower half and in the upper half in all (100%) the 83 adenomas. The number of lysozyme-expressing cells/high power field ranged from 32 to 62 (mean 46.5).

In the normal duodenal mucosa from FAP patients harbouring a duodenal adenoma and in normal subjects, the number of lysozyme-expressing cells/high power field was difficult to assess [6] due to overlapping of the immunological reaction in Paneth cells at the bottom of the crypts.

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 was significant ($p<0.05$). No significant difference was found between FAP and sporadic adenomas.

Discussion

The presence of brightly red, coarse cytoplasmic granules in H&E stain is the single most important parameter that distinguishes Paneth cells [6, 13-15]. In this work, Paneth cells with coarse cytoplasmic granules in H&E were called mature Paneth cells and the lysozyme-expressing cells that exceeded those

found in H&E stain were called precursor Paneth cell precursors. These precursor cells lack the coarse cytoplasmic granules necessary for their detection in H&E sections.

Against that background, two histological-immunohistochemical subtypes of Paneth cells were distinguished: one histologically^{+}-immunohistochemically^{+} corresponding to mature Paneth cells and the other histologically^{-}-immunohistochemically^{+} corresponding to Paneth cell precursors.

Recently, Varnat *et al* [16] showed that peroxisome proliferator receptor β (PPARβ), a nuclear receptor hormone in the GI 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 [16].

Studies on mice indicate that the development and differentiation in the small intestine are under the control of at least 3 signalling pathways known as canonical Wnt, Notch and hedgehog [1]. The canonical Wnt pathway is the key regulator of proliferation of the stem cells in the crypts; its activation is essential to maintain the crypt cell population in a proliferative state. The trans-amplifying progenitors are under the Wnt signal influence domain, whereas differentiated cells, other than Paneth cells, are present in areas where the canonical Wnt signal is inactive. A family of βHLH (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 [1, 17].

Wu *et al* [18] recently demonstrated that Cdc42 (a small GTPase activated by integrins, growth factor, cytokines receptor and cadherins) regulates β-catenin degradation. Cdc42 is important for the terminal differentiation of progenitor cells in the hair follicle of the skin [18]. In the small intestine the nuclear accumulation of β-catenin is mainly observed in cells located at the base of the crypts and decreases as the cell moves toward the top of the crypts [18]. Thus, β-catenin accumulation correlates with areas of cell proliferation whereas β-catenin degradation occurs in areas in which cell differentiation occurs. From these studies it becomes apparent that several molecular signals participate in the regulation of mature Paneth cells and their precursors.

Lysozyme-expressing cells were found haphazardly distributed within the histological profile of the lesion, even in the most superficial cell layer of the dysplastic glands. The latter suggests that, if the innate programmed vector of cell migration were valid for duodenal adenomas (from stem cells towards the bottom of the crypts for Paneth cells), the stem cells, normally overlaying Paneth cells, would have been exfoliated into the lumen. Another less likely option is that mutated stem cells at the bottom of duodenal adenomas translocate, in an unprecedented manner, the ontogenic signals of migration for Paneth cells. This stochastic molecular option would imply a total reversal of the normal migratory vector for Paneth cells, to a more aberrant, paradoxical migration flow, from stem cells to the villus domain, before exfoliation (corrupt migration). Because of the unique migratory direction of the Paneth cells in the crypts of Lieberkühn, the duodenal adenoma emerges as a suitable histo-biological model to monitor the fate of stem cells. It is suggested that stem cells, together with the other recordable mature cells, namely dysplastic enterocytes, Paneth cells and goblet cells, participate in the cell turnover of duodenal adenomas. Further studies are necessary to definitively validate the abovementioned suggested hypothesis.

Please address all correspondences to Carlos A. Rubio, MD, PhD, Gastrointestinal and Liver Pathology Research Laboratory, Department of Pathology, Bldg P1/R8:02, Karolinska Institute and University Hospital, 17176, Stockholm, Sweden. Fax: 46 8 51774524; Email: Carlos.Rubio@onkpat.ki.se

References

[1] Nakamura T, Tsuchiya K and Watanabe T. Crosstalk between Wnt and Notch signaling in intestinal epithelial cell fate decision. *J Gastroenterol* 2007;42:705-710.

[2] Ayabe T, Ashida T, Kohgo Y and Kono T. The role of Paneth cells and their antimicrobial peptides in innate host defense. *Trends Microbiol* 2004;12:394-398.

[3] Sundbom M, Elphick DA, Mahida YR, Cunliffe RN, Midtvedt T, Engstrand L, Rubio CA and Axelsson LG. Alteration in human defensin-5 expression following gastric bypass surgery. *J Clin Pathol* 2007;60:1029-1034.

[4] Ayabe T, Satchell DP, Pesendorfer P, Tanabe H, Wilson CL, Hagen SJ and Ouellette AJ. Activation of Paneth cell alpha-defensins in mouse small intestine. *J Biol Chem* 2002;277: 5219-5228.
[5] Keshav S. Paneth cells: leukocyte-like mediators of innate immunity in the intestine. *J Leukoc Biol* 2006;80: 500-508.
[6] Rubio CA and Nesi G. A simple method to demonstrate normal and metaplastic Paneth cells in tissue sections. *In Vivo* 2003;17:67-71.
[7] Rubio CA. A Paneth cell surrogate? *Gut* 2002; 50:741-742.
[8] Cunliffe R, Rose F, Keyte J, Abberley L, Chan W and Mahida Y. Human defensin 5 is stored in precursor form in normal Paneth cells and is expressed by some villous epithelial cells and by metaplastic Paneth cells in the colon in inflammatory bowel disease. *Gut* 2001;48: 176-185.
[9] Rubio CA, Porwit-McDonald A, Rodensjö M and Duvander A. A method of quantitating Paneth cell metaplasia of the stomach by image analysis. *Anal Quant Cytol Histol* 1989;11:115-118.
[10] Rubio CA. Gastric duodenal metaplasia in duodenal adenomas. *J Clin Pathol* 2007;60: 661-663.
[11] Rubio CA. Serrated adenoma of the duodenum. *J Clin Pathol* 2004;57:1219-1221.
[12] Rubio CA. Do stem cells participate in cell turnover in duodenal adenomas? A preliminary study on Paneth cells. *Anticancer Res* 2008 (in press).
[13] Paneth J. Ueber die secernirenden Zellen des Dünndarms-Epithels. *Arch Mikr Anat* 1888;31: 113-191.
[14] Lewin K. The Paneth cell in disease. *Gut* 1969; 10:804-811.
[15] Odze R, Gallinger S, So K and Antonioli D. Duodenal adenomas in familial adenomatosis polyposis: differentiation of cell differentiation and mucin histochemical features to growth pattern. *Mod Pathol* 1994;7:376-384.
[16] Varnat F, Heggeler BB, Grisel P, Boucard N, Corthésy-Theulaz I, Wahli W and Desvergne B. PPARbeta/delta regulates Paneth cell differentiation via controlling the hedgehog signaling pathway. *Gastroenterology* 2006; 131:538-553.
[17] Shitadshige M, Hirohashi T and Yamada T. Wnt signaling inside the nucleous. *Cancer Sci* 2008;99:631-637.
[18] Wu X, Quondamatteo F, Lefever T, Czuchra A, Meyer H, Chrostek A, Paus R, Langbein L and Brakebusch C. Cdc42 controls progenitor cell differentiation and beta-catenin turnover in skin. *Genes Dev* 2006;20:571-585.