Identification and localization of human intestinal stem cells (hISCs) and expression studies of key regulatory members in homeostasis maintenance

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Introduction

The small intestine is composed of contiguous villi and crypts. Villi consist of three types of mature epithelial cells; the enterocytes responsible for nutrient uptake, goblet cells that produce the protective mucus covering the intestinal epithelium, and enteroendocrine cells that release gastrointestinal hormones.

The crypts are mainly occupied by undifferentiated cells, except for the Paneth cells that are differentiated cells located to the crypt base that secrete antibacterial peptides into the lumen. The epithelium of the colon is similar to that of the small intestine, except for the villi and Paneth cells.

Still, the exact location of the intestinal stem cells (ISCs) has remained controversial. This has primarily been due to the lack of unique molecular markers. However, recent studies have identified a single marker, Lgr5/GPR49, a leucine-rich orphan G-protein-coupled receptor, that specifically labels stem cells in the mouse small intestine as well as other adult tissues (Barker et al., 2007).

In this study, antibodies against Lgr5/GPR49 have been used to localize the predicted intestinal stem cells of the human small and large intestine.

Other suggested ISC markers, as well as established markers for the different cell populations in the epithelium have been investigated. PGE2 is reported to have regulatory effects on stem cell proliferation (North et al., 2007; Kleiveland et al., 2008) and to affect the expression of cyclooxygenase-2 (Cox-2) and the EP receptors were included in the study.

Normal ileum tissue was obtained from right hemicolectomy resections for colon cancer, and normal colon from sigmoidal tissue of patients with rectopexia. Inflamed small intestinal tissue was obtained from patients with untreated coeliac disease.

Results and Discussion

Constitutive Cox-2 expression were detected in few, but evenly distributed cells in the colon crypts. In the small intestine Cox-2+ cells were found close to the crypts, but in the lamina propria. As expected, Cox-2 is highly expressed in the lamina propria of inflamed intestinal tissue.

These results suggest that prostanoids, most likely PGE2, are continuously being produced and secreted in the vicinity of Lgr5+ cells and thus may influence the proliferation and differentiation of putative colon hISCs.

qRT-PCR

Laser microdissection (LMD) were used to isolate epithelial cells for RNA isolation and quantitative real-time PCR. This data was used to confirm the distribution of prostaglandin E2 (EP) receptors within the epithelium of the small and large intestine.

- Our data suggest remarkably different EP receptor usage in the small intestine versus the colon (and inflammatory tissue), most likely reflecting significant differences in EP receptor signaling and function in these tissues.

Our observations add new information to the understanding of the interplay between Cox-2, PGE2 and the EP receptors in the intestinal epithelium, and also suggest paracrine regulatory effects of prostanoids on Lgr5+ intestinal stem cells contributing to homeostatic regulation of vital cells in the human gut.

Main findings:

- Lgr5 shows different expression in colon vs small intestine.
- Cox-2 is found in normal colonic epithelial cells but not in epithelial cell of the small intestine.
- Cox-2 expression is upregulated in lamina propria in inflamed tissue.
- EP receptor expression pattern different in colon compared to the small intestine.

References


References


Figure 1. Lgr5 expression

A-C. Human colon crypt tissue showing positive Hoechst nuclear stain (blue) and single Lgr5-positive cells (green).

D-E. Human small intestine crypt tissue showing positive Hoechst nuclear stain (blue) and Lgr5+ stain in single cells (D=red E=green). Lgr5+ cells are indicated by white arrows, located adjacent to the Paneth cells. The Lgr5+ cells show overall a weaker staining in the small intestine compared to colonic tissue.

The following observations have been made:

- Lgr5/GPR49 and CD133 staining partly coincided in human small intestine and were found directly above the Paneth cells, a position previously suggested to be populated by ISCs.

- This Lgr5/GPR49 staining pattern is different from what has been reported for murine small intestine where Lgr5+ cells were found at the crypt base.

- In human colon crypts Lgr5+ cells were detected at the crypt base, but colon crypts were negative for CD133-expression.

- Provided Lgr5/GPR49 being a marker for hISCs, both based on staining and co-staining patterns, ISCs of the small and large intestine are different and are localized in different positions.

Figure 2. Cox-2 expression

A-B. Picture shows positive anti-Cox2 cells (red) in human normal and inflammatory small intestine crypts, respectively.

C-D. Picture shows positive anti-Cox2 cells (red) in human normal colon crypts.