Identification and localization of human intestinal stem cells (hISCs)

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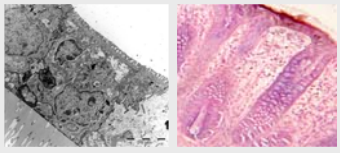

Introduction

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, exept 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).

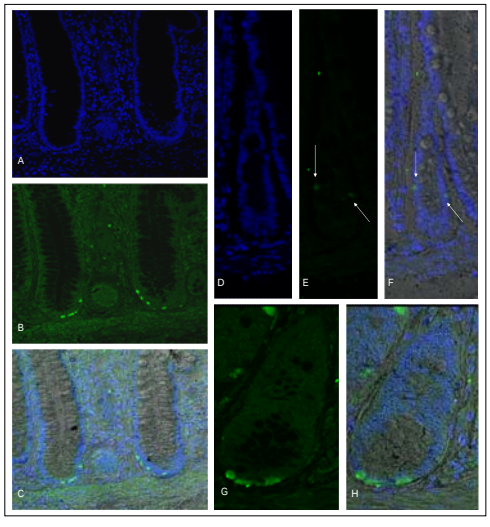

Figure 1.
A-C. Human normal colon crypt tissue showing positive Hoechst nuclear stain (A; blue), single Lgr5-positive cells (B; green) and a merged picture (C) showing nuclear stain and Lgr5+ stain combined in a transmitted light picture.
D-F. Human normal small intestine crypt tissue showing positive Hoechst nuclear stain (D; blue), Lgr5+ stain in single cells (E; green). Lgr5+ cells are indicated by white arrows, located adjacent to the Paneth cells.
G-H. Human normal colon crypt tissue showing Lgr5 positive cells

Results and Discussion

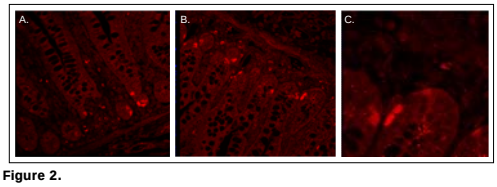

Figure 2.
A-C. Human small intestine crypt tissue showing positive CD133 staining in single cells at the lower part of the crypts.

- Lgr5/GPR49 and CD133 staining coincided in human small intestine, and were found in position +4 from the crypt bottom. This deviates from results reported from murine small intestine.
- In human colon Lgr5+ cells were detected at the crypt base. These cells were however, not CD133 positive.
- Staining and co-localization studies suggest that ISC in the human small intestine and colon are different, and are localized in different positions.
- Constitutive Cox2-expression were detected in cells in the lower part of the human colon crypts. This suggests that prostanoids may influence proliferation and differentiation processes of putative hISCs.
- The staining pattern of the proliferation marker Ki67 show positive cells from the crypt base and crypt walls, declining towards the crypt top/villi.

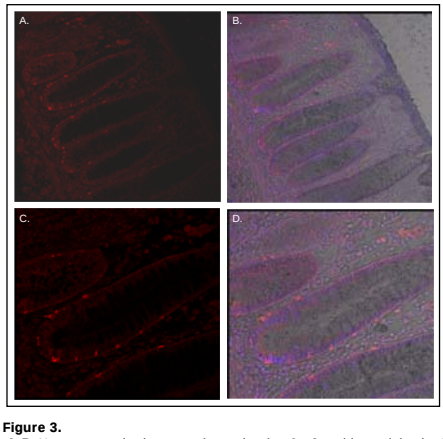

Figure 3.
A-B. Human normal colon crypt tissue showing Cox2 positive staining in single crypt cells (A,red). The merged picture (B) show nuclei Hoechst stain (blue), positive Cox2 stain (red) combined in a transmitted light picture.
C-D. Human normal colon crypt tissue showing Cox2 positive staining

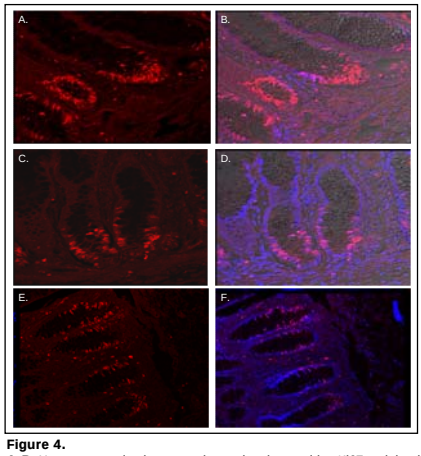

Figure 4.
A-B. Human normal colon crypt tissue showing positive Ki67 staining in single crypt cells (A, red). The merged picture (B) show nuclear Hoechst stain (blue) and positive Ki67 stain (red) combined in a transmitted light picture.
C-D. Human normal colon crypt tissue showing positive Ki67 staining in single crypt cells in the basal part of the crypt. No Ki67 positive cells are found in the extreme basal part of the crypt (indicated by white arrows), known as the stem cell region.
E-F. Human small intestine crypt tissue showing Ki67-positive staining in single crypt cells in the basal part of the crypt.

Future Perspectives

- Characterization of the transcriptome of Lgr5+ cells in normal and inflamed intestine:

We have recently established protocols for laser microdissection of single cells from intestinal tissue (Leica LMD). Subsequently, we will isolate mRNA from the samples allowing us to carry out an analysis of the gene expression profile (Affymetrix).

- Quantitative studies of the major epithelial cell populations in normal and inflamed intestine

- Immortalization of Lgr5+ cells by retroviral transduction with a telomerase reverse transcriptase (TERT) gene construct.

To be able to study the biology of intestinal stem cells in vitro, we will use immunomagnetic cell separation (MACS system, Miltenyi Biotech) as a tool to isolate Lgr5+ cells from enzyme-digested mucosal tissue.

- Characterization of the secretome of Lgr5+ cells

The cytokine and chemokine secretion profile of TERT-transduced Lgr5+ cells will be studied by a multiplex fluoroimmunoassay technique.

- Characterization of intracellular signalling pathways in Lgr5+ cells

The gene expression studies will be supported by a comparative characterization of the activity in the most relevant intracellular signalling pathways known to be involved in regulating stem cell proliferation and differentiation, i.e. the Wnt pathway, the Hedgehog pathway, the Notch pathway and the BMP pathway.

References

Barker N, van Es JH, Kuipers J et al. Identification of stem cells in small intestine and colon by marker gene Lgr5. *Nature* 449, 1003-1007, 2007