

## Putative intestinal stem cells

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### Abstract

A heterogeneous set of intestinal stem cells markers has been described in intestinal glands but the ultrastructural identity of intestinal stem cells remains unknown. By using electron microscopy, this study demonstrated the presence of cells with stem morphology in the intestinal glands of mice of different ages. These putative intestinal stem cells have large, euchromatic, irregular shaped nucleus, large, visible nucleolus, few ER cisternae and mitochondria. Their morphology is distinct from the morphology of any other intestinal gland cell. Stem cells located at the base of intestinal glands undergo mitosis. This study enhances the hypothesis of a gland (crypt) base columnar cell that gives rise to all the intestinal lineages.

**Keywords:** intestinal stem cells, stem cell ultrastructure, intestinal stem cell niche

### Introduction

The ability to regenerate and replace cells is vital for the viability and homeostasis of most epithelial tissues, including the intestinal tract. Cellular regeneration typically depends on stem cells: primitive and relatively unspecialized cells in fetal and adult tissues that have properties of self-renewal, clonogenicity and multipotency [1].

The presence of adult stem-like cells in the gastrointestinal tract was first postulated by Charles LeBlond 60 years ago [2], before they were recognized in other organs. Adult stem cells, such as intestinal tissue stem cells, lack cell specific patterns of expression but give rise to the so-called progenitor cells. These, in turn, produce cellular descendants that have a more restricted lineage potential [3]. There is an ongoing debate about how many intermediate cell entities, such as progenitor cells, exist [4].

Stem cells in the intestine are located in specific sites within the epithelium, adjacent to areas of rapid proliferation and high cell turnover. Proliferation occurs at the base of intestinal crypts in the small intestine; most of the cells migrate up from the crypts to the villi, while some of the cells migrate below the stem cells to form Paneth cells. A few enteroendocrine, mucus and columnar cells might also migrate downward from the common origin into cell positions 1–4 [5].

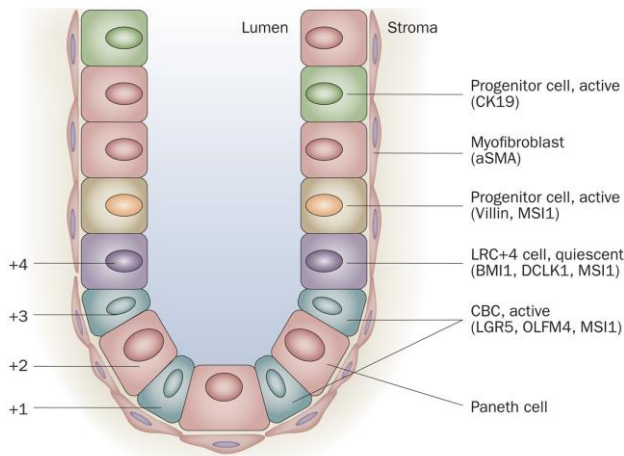
In 2007, a single marker, LGR5, a leucine-rich orphan G protein-coupled receptor, was identified in lineage-tracing studies to specifically label stem cells in the mouse small intestine, such as the crypt base columnar cells between the Paneth cells [6]. This research has reactivated the debate about the location of intestinal stem cells. Some LGR5-positive cells seem to be multipotent and are able to form all mature intestinal epithelial cells. They seem to undergo self-renewal, to

persist for several months and to be resistant to irradiation. Thus, these rapidly proliferating cells with intestinal stem cell characteristics have challenged the previously held belief that all adult stem cells are generally quiescent or slowly cycling [7].

In 2009, lineage-tracing studies of adult prominin-1 (also called CD133; a pentaspan transmembrane glycoprotein that localizes to membrane protrusions) showed that some prominin-1-positive cells are located at the base of crypts in the small intestine, co-express LGR5 and can generate the entire intestinal epithelium, and therefore seem to be small intestinal stem cells as well [8,9].

**Table 1.** Intestinal tissue stem cell markers

Marker	Characteristics of cells
LGR5	Active cycling crypt base columnar cells that give rise to all intestinal lineages (lineage tracing) [6]
Prominin-1	Active cycling crypt base columnar cells that give rise to all intestinal lineages (lineage tracing), overlaps with LGR5 [8-10]
BMI1	Quiescent cells around position 4+ that give rise to all intestinal lineages (lineage tracing) [11]
DCLK1	Expression around position 4+ (no lineage tracing) [12,13]
CCK-BR	Probably present on, but not specific for colonic stem cells or progenitor cells [14]
Label retaining (BrdU)	Quiescent cells at position 4+ [15]



**Fig. 1** Schematic illustration of the location of putative intestinal stem cells and/ or progenitor cells and their markers in the crypt of the intestine. Quiescent stem cells may be located at position +4, the more active stem cells (crypt base columnar cells [CBCs]) are located anywhere from position +1 to +4 scattered between the Paneth cells. The intestinal glands are surrounded by stromal cells (niche cells), such as myofibroblasts. Quante M, Wang TC. Physiology. Bethesda. 2008; 23, 350–359

This paper tried to identify the putative intestinal stem cells in their stem cell niche, intestinal cells progenitors and their morphology in different developmental stages, by electron microscopy, from two weeks to adulthood in mice, in a comparative study with the literature data.

The features of putative intestinal stem cell are not yet known and their ultrastructural phenotype(s) should be of great interest for their characterization.

## Materials and Methods

### Transmission electron microscopy

Small tissue fragments (about 1mm<sup>3</sup>) from mouse intestine were fixed in 4% glutaraldehyde solution (in 0.1M cacodylate buffer), prepared fresh for 4 h at 4°C. After a brief wash of the samples in 0.1M sodium cacodylate the solution was followed by a step of postfixation at room temperature for 60 minutes in a mixture of 1% potassium ferrocyanide and 1% osmium tetroxide in 0.05 M sodium cacodylate buffer (pH 7.4).

Samples were then dehydrated in solutions with increasing ethanol concentrations. After impregnation of propylene, the tissue was immersed overnight in a mixture of propylene oxide and resin Epon 812 and Epon included in the section has been made ultrafine (50 nm), by using ultramicrotome MT 7000 (Research Manufacturing Company, Inc., Tucson, AZ, USA), after

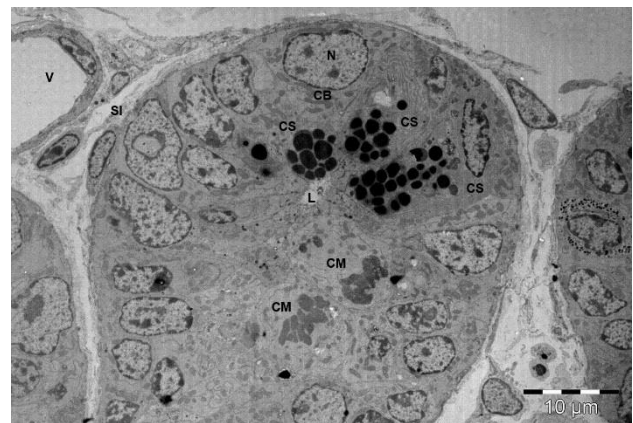
which they were mounted on copper grids and contrasted with uranyl acetate and Reynolds' lead citrate.

Digital images were taken with MegaView III CCD camera, operated by ITEM- the SIS software (Olympus Soft Imaging System GmbH, Germany) and transmission electron microscope mounted Morgagni 286 TEM (FEI Company, Eindhoven, The Netherlands) at 60 KV.

## Results

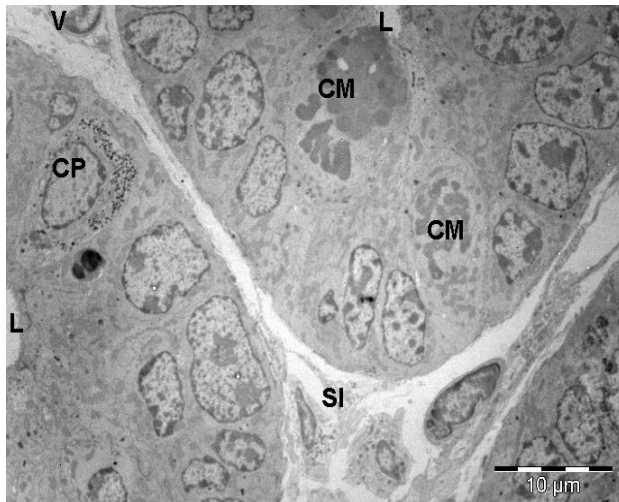
While using electron microscopy and exclusion criteria, it was found that some intestinal epithelial cells presented ultrastructural features of stem cells. These putative intestinal stem cells have been found in specific areas of the epithelium, adjacent to the rapidly proliferating area.

Transmission electron microscopy (Fig. 2) showed a cross section through a Lieberkuhn gland from small intestine of a two-week old mice, in which two dividing cells could be seen near the lumen, considered according to literature precursor cells and at the basis of the gland, besides Paneth cells, cells with ultrastructural appearance like young cells: large nucleus (core report/ cytoplasm above par), euchromatic, visible nucleolus and cytoplasm with few organelles: the mitochondria and endoplasmic reticulum few tanks, considered to be stem cell, corresponding to literature data as gland position (Fig. 1).



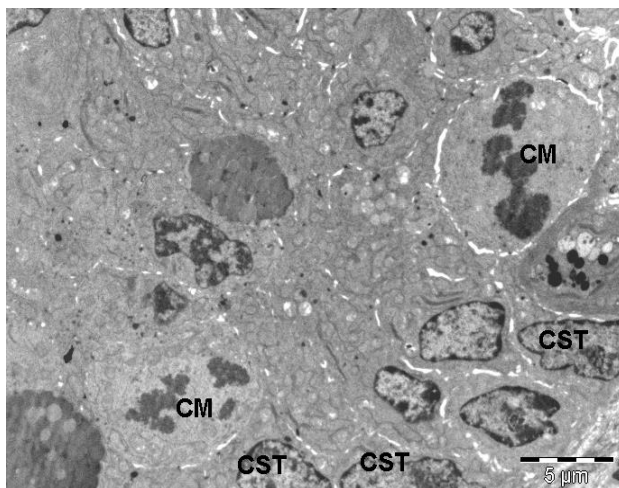
**Fig. 2** Transmission electron microscopy image of the basal area of the Lieberkuhn gland of the small intestine (two-weeks-old mouse). Cells with morphology of stem cells were observed at the base of Lieberkuhn gland of the intestinal mucosa along with secretory cells and two cells in mitosis in a higher floor. This image shows putative intestinal stem cells in intestinal stem cell niche. L-intestinal lumen; CM-cells in mitosis; N- nucleus; CB-basal cell; CS-secreting cell; SI-interstitial space; V-blood vessel

Cells presented in this paper as putative stem cells or progenitor cells have been found to undergo mitosis (Fig. 2).

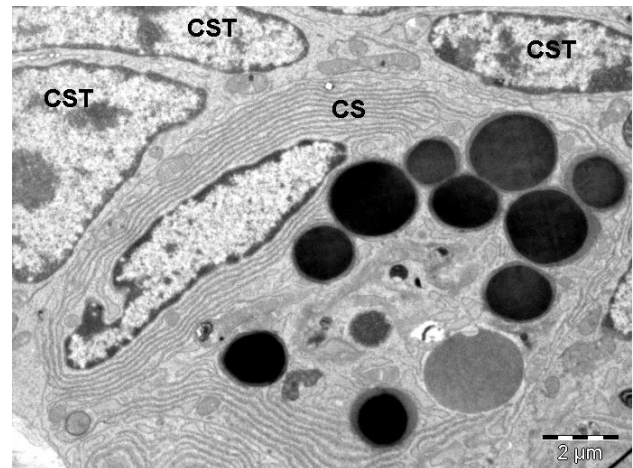


**Fig. 3** Transmission electron microscopy image of basal areas of Lieberkuhn glands of the small intestine (two-weeks-old mouse). Cells with morphology of stem cells were observed at the base of Lieberkuhn gland of the intestinal mucosa along with a Paneth cell and two cells in mitosis in different floors. L-intestinal lumen; CM-cells in mitosis; N-nucleus; CP-Paneth cell; SI-interstitial space; V-blood vessel

Putative intestinal stem cells or cells with stem cell morphology or basal cells are cells with large, euchromatic, irregular shaped nucleus, large nucleolus, few endoplasmic reticulum cisternae and mitochondria.



**Fig. 4** Transmission electron microscopy image showing small intestine epithelium (three-months-old mouse). Cells with morphology of stem cells were observed between the other epithelial cells. CM-cells in mitosis; CS-cells with morphology of stem cells



**Fig. 5** Transmission electron microscopy image showing small intestine epithelium (one-year-old mouse). Cells with morphology of stem cells were observed between the other gland cells. CST- cells with morphology of stem cells, CS-secreting cell

## Discussion

Tissue-restricted stem cells are generally difficult to identify morphologically and are not easily distinguished from other epithelial cells by any consistent set of markers, except for perhaps their ability to divide and self renew [16,17]. Tissue stem cells or progenitor cells are thought to reside within a “niche”—an area with extracellular substrates that provide an optimal microenvironment for normal differentiation. Progenitor cells divide quickly and are responsible for the bulk of cell division, but seem to have a limited lifespan and are replaced periodically by descendents of the true stem cell [18].

Studies in transmission electron microscopy have shown that in different sections, the cell with the particular morphology does not resemble any other intestinal epithelial cell morphology, but are young cell-like cell morphology, undifferentiated or stem cells.

The location of these cells was consistent with the location of the stem cell markers in literature data. In comparison, a decrease in mitosis was observed to be present in the intestinal epithelium with age, most present on the pictures taken from the intestine of two-weeks-old mice.

The next step would be to identify the stem cell markers on electron microscopy studies along with the stem cell microenvironment or niche and with the signals that regulate the behavior of these stem cells.

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