CELL TECHNOLOGIES IN BIOLOGY AND MEDICINE

Stem Cells of Small Intestinal Epithelium

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Translated from *Kletochnye Tehnologii v Biologii i Meditsine*, No. 2, pp. 63-67, April, 2008
Original article submitted December 6, 2007

Intestinal epithelium is a rapidly proliferating tissue and represents a unique model for the study of somatic stem cells. Here we summarized the data about markers, main properties, and methods of isolation of intestinal epithelial cells. Some aspects of molecular biology of the intestine are also discussed.

Key Words: *epithelium, small intestine; stem cells; differentiation; regulation*

The study of stem cells (SC) of the small intestine was started more than 30 years ago, when in 1974 H. Cheng and C. Leblond hypothesized that non-differentiated cells located near the bottom of the crypts give rise to various cell populations of the small intestinal epithelium [10]. Study in the field of the small intestine biology plays now an important role in the development of effective regenerative therapy of intestinal lesions and in short intestine syndrome.

A critical problem is the absence of markers of SC of the small intestinal epithelium allowing identification of these cells [8,10,27]. Nevertheless, new attempts are undertaken to isolate these cells from the cell mass of the regenerative zone of the intestinal crypts. For instance, C. Potten and T. Kayahara obtained interesting data on the expression of Musashi-1 (Msi-1) gene encoding an RNA-binding protein of intestinal SC [28]. It should be noted that Msi-1 protein participates in asymmetrical division of nerve SC [24]. Immunohistochemical study and *in situ* hybridization showed that only small amount of Msi-1 is normally detected in the SC zone of intestinal crypts. After irradiation, expression of Msi-1 in the clonogenic region increases, which demonstrates the possibility of using this protein as a marker of functional SC of the intestine [28]. Msi-1 also participates in the regulation of the expression of transcription molecules Hes-1 playing an important role in self-maintenance and suppression of SC differentiation [19].

Unfortunately, traditional methods of SC isolation based on the use of surface markers and the corresponding antibodies cannot be applied for intestinal SC because of the lack of specific markers [30]. That is why C. Dekaney and co-workers proposed a method called SP sorting for this purpose [10]. This method is based on absorption of fluorescent DNA-binding dye Hoechst 33342 by cells and was initially described for hemopoietic SC [12]. Dual color flow cytofluorometry of bone marrow cells stained with Hoechst 33342 revealed a distinct subset of cells, so-called side population (SP). Analysis of SP cells from the mouse bone marrow by tradition methods based on the detection of surface markers showed that SP is a population of hemopoietic SC [13]. Further experiments showed that SP-phenotype characterizes SC of the esophagus, skeletal muscles, myocardium, mammary gland, and liver [4,5,23,25,39]. In experiments of C. De-

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0007-4888/08/1454-0495

kaney *et al.* with co-workers, cell marker CD45 was used for separation of the hemopoietic and non-hemopoietic fractions of SP cells from the small intestine. It was assumed that CD45-negative fraction of SP cells comprises epithelial SC of the intestine. This hypothesis is based on findings that this fraction is enriched with Msi-1, a marker of intestinal SC. It was found that CD45-negative SP fraction of small intestinal cells is characterized by low expression of markers typical of other SC. The presence of β_1-integrin (87% in CD45-netgative SP fraction), a surface marker detected also on esophageal and colon SC, suggests that SC of the gastrointestinal tract possess similar characteristics distinctive from other tissues [10].

On the basis of the total number of epithelial cells lining the crypts and villi of mouse small intestine, C. Dekaney *et al.* calculated the quantitative ratio of SC in the whole epithelium: 0.1-0.5%. The authors also showed that CD45-negative SP-fraction constitutes 2.1±0.6% living cells of the epithelial preparation. Thus, the number of cells in this fractions is higher than theoretically predicted for the pure preparation of primitive intestinal SC. Hence, this population is heterogeneous and includes not only SC, but also considerable amount of transitory precursor cells. This conclusion is based on the fact that CD45-negative fraction of small intestinal cells was depleted by enterocyte marker (sucrase-isomaltase), but enriched with goblet cell and Paneth's cell markers (intestinal trefoil factor and lysozyme) [10]. Goblet cells and Paneth's cells start differentiating in the transitory zone, whereas enterocytes express differentiation markers (*e.g.* sucrase-isomaltase) only when they attain the site of crypt/villus junction [10]. Further studies are required for detection of markers of intestinal SC.

Despite difficult procedure of isolation of SC from the intestine, their study was based on functional tests for the recovery of the epithelium after damaging exposures [8,27]. The use of this approach made it possible to demonstrate the presence of actual SC, potential SC (most likely, the closest descendants SC of actual SC), and transitory precursor cells divided by mitosis and differentiating into a certain type of epitheliocytes [21,26].

Actual SC are highly sensitive to DNA damage induced by γ-irradiation and occupy position 4 in the crypts of Lieberkühn (4 cells up from the crypt bottom). The exact number of true SC in each crypt is an open question. Many authorities hypothesize that each crypt contains 4-6 SC [26], while some investigators report that only one SC is present in the crypt [9,14].

Apart from actual SC, the intestine contains potential SC, which can function as true SC, if necessary (*e.g.* after damage) [27]. It was demonstrated that each crypt of the small intestine in mice contains 30-40 clonogenic potential SC less sensitive to γ-irradiation and about 125-150 cells, which can divide, but lack self-maintenance and crypt regeneration capacities [7,8,27]. Precursor cells also reside in the crypts and during differentiation migrate to the top of the villus, where mature cells uncapable of division die and are desquamed. The total cycle of epitheliocyte renewal is 2-3 days in mice and 5-6 days in humans [29]. Thus, the epithelium of crypts and villi is an integral system consisting of cell subsets at different differentiation stages.

Among biological characteristics of small intestinal SC, the most important are their capacity to self-maintenance, proliferation, and differentiation.

The self-maintenance capacity (long-term reproduction of the same cells) or proliferative potential is the first principal property of SC. This characteristic reflects the capacity of SC to maintain the size of population via cell division. This division can be symmetrical and asymmetrical. Asymmetrical division yields 2 cells: one daughter cell retains SC status, while other cell continues dividing and enters differentiation pathway. Symmetrical division yields either two SC or two differentiating cells (SC disappears) [20].

Another principal property of SC is its differentiation capacity. Intestinal SC are multipotent cells, *i.e.* can give rise to at least 5 phenotypically and functionally different populations of intestinal cells: Paneth cells, enteroendocrine cells, goblet cells (cells producing mucin), M cells, and columnar epithelial cells [27].

The epithelial lining of the crypts consists primarily of columnar epithelial cells. These are prismatic cells with thinner striated border and basophilic cytoplasm. Pronounced polar structure of these cells reflects their functional specialization, *i.e.* resorption and transport of nutrients [31].

Epithelial cells lining the areas occupied by Peyer patches drastically differ by their structure and function from other enterocytes. Microvilli on their apical surface are shorter and wider than microvilli on columnar epitheliocytes and resemble microfields, that is why these cells were called M-cells. M-cells closely contact with lymphocytes, macrophages, and plasma cells. The function of M-cells is well studied. These cells capture and transport antigens from the intestinal lumen to the lymphoid cells, thus enabling antigenic stimulation of the local immune system of the small intestine [32].

Goblet cells also constantly reside in crypts. Structurally, these are typical mucous cells undergoing cyclic changes related to accumulation and secretion of the mucus. The mucus produced by goblet cells moistens the mucosa, thus promoting the passage of nutrient particles [11].

Paneth cells are located at the bottom of the crypts. Paneth cells are characterized by high content of zinc, selenium, and enzymes (acid phosphatase, dehydrogenases, and dipeptidases). The presence of some enzymes in these cells attests to participation of products secreted by these cells in the digestion processes. Antibacterial function related to lysozyme production (the enzyme destroying bacterial and protozoon cell walls) is also very important. Thus, Paneth cells play an important role in the regulation of bacterial flora in the intestine [22,27].

Endocrine cells are more abundant in the crypt than in the villus. EC-cells secreting serotonin, motilin, and substance P predominate. A-cells producing enteroglucagon are scanty. S-cells producing secretin are irregularly located in various portions of the intestine. I-cells secreting cholecystokinin and pancreozymin, bioactive substances stimulating the functions of the pancreas and liver, were found in the intestine. G-cells producing gastrin and D- and D1-cells producing active peptides (somatostatin and vasoactive intestinal peptide) were also found [15].

Stem cell niche is a cornerstone of the molecular biology of SC. According to the hypothesis of unique microenvironment (niche), the crypt bottom contains various factors regulating cell cycle of SC, their self-maintenance, proliferation, differentiation, and migration. This is an ideal environment for the maintenance of SC phenotype. The regulation of SC functions is the basis for interaction of several signal pathways, the most important of them are BMP (bone morphogenetic protein), Hedgehog, Wnt, and Notch [34,38].

Wnt—Frizzled—β-catenin signal pathway plays a key role in the regulation of asymmetrical division of cells.

Under conditions of normal growth in the absence of Wnt-signal pathway, intracellular β-catenin is phosphorylated by the serine residue under the effect of a destruction complex, which maintains the content of cytosolic β-catenin at a low level [20]. The interaction of Wnt-ligand with the corresponding receptor triggers the signal cascade leading to accumulation of cytosolic β-catenin. β-Catenin binds Tcf-4 (T-cell factor-4) and acts as its co-factor, thus activating transcription and expression of the target genes responsible for cell proliferation [6,20].

Signal pathway Notch is responsible for differentiation of multipotent intestinal SC. High levels of Notch protein inhibit transcription of Math-1gene via transcription repressor Hes-1 [20]. Low levels of Notch protein are associated with accumulation of Delta ligand, which blocks Hes-1 and triggers the expression of Math-1; these processes lead to differentiation into Paneth cells, goblet cells and enteroendocrine cells [18,20].

The BMP signal pathway plays an important regulatory role. After binding with the corresponding receptor, it transmits the signal through SMAD transcription factors [20]. Moreover, the BMP signal pathway can prevent the self-maintaining activity of the cell by inhibiting β-catenin and suppressing Wnt signal pathway [17,20].

Expression of Hh genes (sonic hedgehog — Shh, Indian hedgehog — Ihh) plays an important role in proliferation and differentiation of epithelial SC. In Ihh-knockout mice, the size of villi is reduced and proliferation of SC in the niche is low [29,35]. Moreover, Wnt and Ihh can regulate each other. Thus, treatment with cyclopamine (an inhibitor of the Hedgehog signal pathway) leads to positive regulation by Wnt of the target genes (engrailed-1, cyclin D1, Bmp-4) in the SC niche. These studies suggest that Ihh can restrict Wnt-sensitive cells residing in the SC compartment [29,33].

Apart from such principal characteristics as self-maintenance, proliferation, and differentiation into epithelial cells, SC are characterized by the capacity to form differentiated cells of other tissue [16,36].

Thus, culturing of mouse and human small intestinal epithelium on a feeder layer (fibroblasts) led to the formation of a new type of intestinal cells expressing nestin [36]. *In vitro* experiments showed that intestinal cells expressing nestin (an indicator of multipotent and regeneratory potential of cells) exhibited high proliferative activity and differentiated into cells expressing markers of the nervous, liver, and pancreatic tissue. However, these derived cell were functionally inactive. It was also shown that embryonic fibroblasts are characterized by intensive expression of Wnt/BMP, and the formation of nestin-positive intestinal cells is associated with high level of Lef1, Wnt4, Wnt5a, and Wnt/BMP-factors and reduced content of BMP-4. C. Wiese with co-workers hypothesized that the use of embryonic fibroblasts leads to re-programming of adult intestinal epithelial cells via modulation of Wnt/BMP signals into cell type with more primitive embryonic-like developmental status, which ensures higher degree of plasticity [36].

In the study of D. He, intestinal SC from ROSA26 mice expressing LacZ transgene were isolated by

flow cytofluorometry and by the method of SP sorting [10,12] and injected into small intestinal submucosa of irradiated C57Bl/6 mice. It was found that recipients of the experimental group had less pronounced skin lesions and the color of the hair on irradiated parts of the body remained practically unchanged compared to controls. Histochemical studies showed that donor cells could give rise to skin cells in irradiated recipients. These experiments demonstrated that SC from the small intestine can differentiate into skin cells under certain conditions [16].

It was also shown that transplantation of the epithelial cells from the small intestine to lethally irradiated recipients leads to the formation of splenetic colonies [3]. Using genetic and histological methods, we found that these colonies are hemopoietic and represent clones of donor cell [2]. Moreover, the cell population of the peripheral blood and functional activity of immune system cells were restored 6 months after transplantation of the small intestinal epithelial cells to lethally irradiated recipient.

Thus, small intestinal epithelium is a complex and multicomponent system and is an interesting object for investigation, because it is a unique model for the study of SC.

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