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Review

Localization of Stem Cells in Small Intestinal Epithelium : Strategies for Identifying Small Intestinal Stem Cells

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SUMMARY
In the small intestine, stem cells are considered to exist at the bottom of the crypt. Actively proliferating transitional cells supplied from stem cells are differentiated into two directions upward and downward. Upward cells are differentiated into absorbing epithelial cells, goblet cells, and endocrine cells, and downward cells differentiated into Paneth cells. However there are some difficulties to identify the stem cells because of their unique characteristics. At first, stem cells occur as actual stem cells and potential stem cells, and second, there is diversity in stem cells. Therefore, molecules suitable for a marker of small intestinal stem cells are necessary to distinguish "true" stem cells from others. Energetically searched for in recent years, Musashi-1, type 1A bone morphogenetic protein receptor (BMPR-1A), phospho-phosphatase and tensin homolog deleted on chromosome ten (phospho-PTEN), doublecortin and calmodulin kinase-like-1 (DCAM-KL1), ephrin receptors (Eph receptors), integrins, and leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5) are proposed. Among them, Musashi-1 draws attention as one of a candidate marker for small intestinal stem cells. We here introduce our reviews about expression of Musashi-1 and Hes1 proteins in the small intestine, and would like to overview the way to identify the small intestinal stem cells.

Key Words : stem cell, small intestine, Musashi-1, Hes1, crypt base columnar cell

INTRODUCTION

The small intestine is morphologically characterized : villous cells are supplied from stem cells at the crypt base. Namely, undifferentiated to differentiated villous cells are lined in sequence on the villus, indicating that the small intestinal epithelium could be one of candidate organs suitable for stem cell research. However, the mechanism hasn't been elucidated yet, since stem cells have been variously identified among research. Then, based on a fact that Musashi-1 and Hes 1, neural stem cell markers, are expressed in the small intestinal epithelium, we found a possibility that these proteins can be markers of stem cells in the small intestinal epithelium.

In this article, the authors would like to comment on previous research involved in identification of stem cells in the small intestinal epithelium, including overviews of these markers, and describe the directions of researches in future.

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Small intestinal stem cells reside at the crypt base

The small intestine is an organ where ingested foods are digested and absorbed. This means that this organ always contacts with the external environments, being

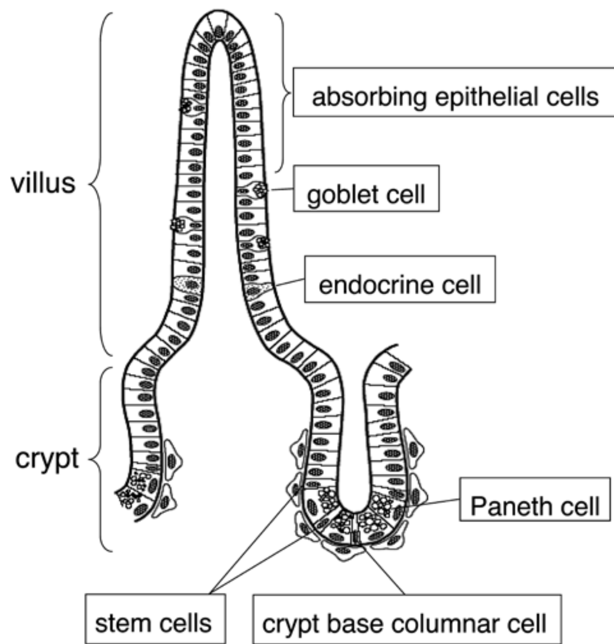

Fig. 1 Structure of small intestinal epithelium
Actively proliferating transitional cells supplied from stem cells at the crypt base are differentiated into two directions upward and downward. Upward cells are differentiated into absorbing epithelial cells, goblet cells, and endocrine cells while upward moving on a villus. On the other hand, downward moving cells differentiated into Paneth cells.

exposed to hazards. Therefore, intestinal cells are very rapidly turned over, being momentarily replaced by new generations of cells. In addition, the small intestine is also known as an organ whose regeneration is extremely rapid when damaged. The small intestinal epithelium is continuously renewed at intervals of 2 to 7 days by perpetually producing new cells[1~7]. This production of new cells is achieved by stem cells.

Although there are various opinions explaining cellular regeneration and renewal in the small intestinal epithelium, the following is an almost consolidated explanation : actively proliferating transitional cells supplied from stem cells at the crypt base are differentiated into two directions upward and downward. Upward cells are differentiated into absorbing epithelial cells, goblet cells, and endocrine cells while upward moving on a villus. When reaching the villous end, cells induce apoptosis to deciduation into the intestinal lumen. On the other hand, downward moving cells differentiated into Paneth cells at the crypt base are likely to die and be phagocytized by adjacent crypt base columnar (CBC) cells (Fig. 1)[1,5~9].

Meanwhile, how could small intestinal stem cells be recognized to be present at the crypt base? For one of the reasons, a fact can be cited that there are more actively dividing cells at the crypt base than at the villus. This has long been estimated that cells in the crypt are divided and migrate toward the villus[2]. In addition, a pulse-chasing method following up DNA precursors labeled with 14C-adenine or 3H-thymidine with time was able to detect how labeled cells in the crypt migrate to the villus[3,4]. On the other hand, CBC cells located at the crypt base are known to embrace phagozomes which phagocytized dead Paneth cells, and the phagozomes can be observed by labeling with 3H-thymidine. These labeled phgozomes can be detected even in absorbing epithelial cells, endocrine cells, goblet cells, and Paneth cells with time[1]. This means that the four cell lines differentiating upward and downward are supplied from CBC cells located at the crypt base. These facts definitely suggest that multipotential cells reside at the crypt base. In this connection, in some studies, the migration speed of cells from the crypt to villus was calculated to estimate the originating point of migration, advocating that 4th to 5th cells from the crypt bottom could be stem cells[10,11].

There is a hypothesis that old DNA templates selectively move to stem cells while cells are dividing, as a means for stem cells to circumvent DNA mutation during replication[12,13]. According to this hypothesis, thymidine and BrdU incorporated into chromosomes during DNA replication are diluted at every cell division. However, cells continuously carrying such labels represent those selectively preserving once replicated DNA, in other words, stem cells. Cells procured by this method are called label-retaining cells (LRC), being used as a marker of stem cells. Mouse small intestinal LRC, pulse-labeled as above, is located at 4th to 5th cells from the crypt bottom, i.e., immediately above Paneth cells[14]. The location coincides with that mentioned above, and those cells are considered to be a strong candidate as small intestinal stem cells. On another front, however, homologous recombination of DNA frequently occurs during DNA replication for cell division to equally deliver the replicated DNA, and thus, this hypothesis is often questionable. Further-

more, LRC is observed only when the cells are labeled after the small intestine is exposed to radiation, and therefore, it is also considered that actual stem cells may be already at the stage of apoptosis under this condition, as mentioned below, indicating that the labeled cells are not actual stem cells but potential stem cells.

Stem cells occur as actual stem cells and potential stem cells

In general, stem cells refer to a group of cells which are capable of both self-replication and differentiation into various types of functional cells. In addition, stem cells are considered to be most predisposed to apoptosis, so as not to inherit DNA damages. In the small intestine, there exist cells which can displace the damaged stem cells. However, whether or not such cells should be included in a category of stem cells has made it ambiguous how to define small intestinal stem cells, giving rise to the confusion. Then, Potten et al.[14,15] proposed a concept that tissue stem cells are divided into two categories : actual stem cells assuming constitutive maintenance of the constant tissues and potential stem cells which can be replaced when they are damaged.

Some reported a study in which the number of actual stem cells and potential stem cells were estimated by measuring surviving or regenerating cells under changed radiation doses[15,16]. Actual stem cells are considered to be easily killed at a low radiation dose, and these 4 to 6 actual stem cells exist in a crypt. This number can just supply about 250 cells present in a crypt through 6 generations[15,17]. Although a low dose of radiation decreases the number of actual stem cells, cell cycle is transiently accelerated in a region where potential stem cells are present, seemingly soon restoring the decreased number of actual stem cells. This allows a hypothesis that the number of actual stem cells is strictly regulated[15,17]. In contrast, 30 to 40 potential stem cells exist in a crypt, which are resistant to increased doses of radiation, allowing regeneration of crypt through monoclonal proliferation[15].

By the way, what is a difference between actual stem cells and potential stem cells? It is not known yet, whether actual stem cells bear their inherent properties or they passively acquire the properties depending on surrounding microenvironments able to maintain the stem cells.

There is diversity in stem cells which constitute a crypt

The small intestine is constituted in the postnatal development stage. In neonatal mice, villus already exists but crypt does not. Around postnatal day 7, depression occurs among villi, where crypt starts to be formed. Simultaneously Paneth cells also sporadically appear at the crypt base. Around postnatal day 21, the crypt is completed, appearing as morphologically same as that in adult animals. In analyses using chimeric mice, stem cells were shown to be polyclonal when the crypt started to be formed, but turned monoclonal when the crypt is completed[18].

On the other hand, whether stem cells existing in a single crypt are characterized by a single nature is questionable. In pursuit of differentiation of stem cells in a small intestinal cell linage which randomly mutate, it was found that there are two differentiation pathways of stem cells : one into short-lived progenitor cells which can be differentiated into either of columnar epithelium and mucus-producing cells and another into long-lived progenitor cells whose differentiation is directed to one of those cells. The short-lived progenitor cells can differentiate into progenitor cells of columnar epithelium and mucus-producing cells in unit of day, but the long-lived progenitor cells persistently stay at the crypt base in unit of month, supplying progenitor cells through asymmetric division[19]. This is an example that there is diversity even in genealogy of stem cells.

Musashi-1 is one of a candidate marker best suitable for small intestinal stem cells

Molecules suitable for a marker of small intestinal stem cells have been energetically searched for in recent years, and Musashi-1, BMPR1alpha, phospho-PTEN, DCAMKL1, Eph receptors, integrins and Lgr5 are proposed[20]. Among them, Musashi-1 which is a marker of neural stem cells draws attention as one of a candidate marker for small intestinal stem cells[21,22]. We here introduce our reviews about expression of Musashi-1 and Hes 1 proteins in the small intestine and their roles.

Musashi-1 was originally isolated and identified as a

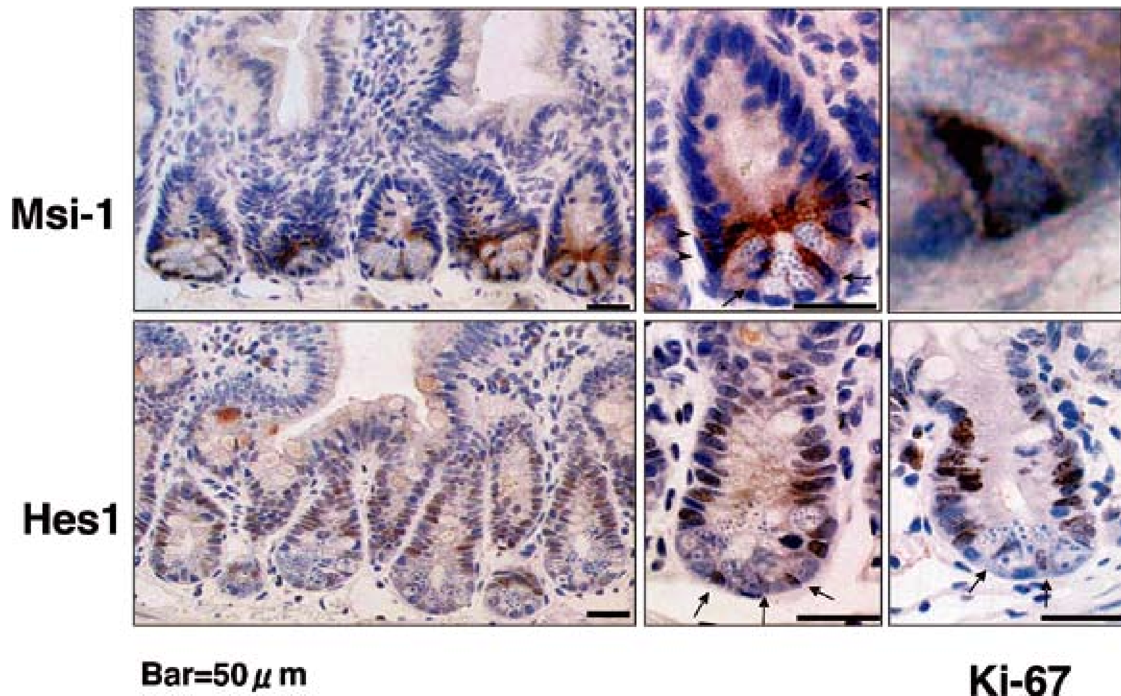

Fig. 2 Immunohistochemical analysis of Msi-1, Hes1, and Ki-67 in adult mouse small intestine. A few crypt cells just above the Paneth cells (arrowhead) and the crypt base columnar cells between the Paneth cells (arrow) were stained with Msi-1 antibody. Hes1 was predominantly expressed in mid to lower crypt cell nuclei and immunoreactivity gradually increased toward the crypt base. Crypt base columnar cells were also stained with Hes1 and Ki-67antibody (arrow). (Cited from Kayahara T et al, 2003[20])

protein expressed by a gene causative of mutant drosophila bearing anomalous external sensory organs[23]. This protein molecule is abundantly expressed even in stem cells of mammal NCS[24], which has been reported to be involved in development of neurons and glias[25]. Musashi-1, a protein binding to RNA at two sites, is considered to repress expression of a protein at translation level by binding to its mRNA[26]. On the other hand, Hes 1 is known as a basic helix-loop helix type repressive transcription factor, which plays a role as downstream effector in Notch signaling. Hes 1 is related to repression of neural differentiation in the CNS[27], which is considered to inhibit Math, Mash, and Neurogenin that are transcription factors to promote differentiation[28]. In the enteric canal of Hes 1-knockout mice, differentiation into endocrine cells, goblet cells and Paneth cells are facilitated[29,30], and inversely in Math 1-knockout mice, differentiation into endocrine cells, goblet cells, and Paneth cells are suppressed[31].

As for relationship between Musashi-1 and Hes 1, a working hypothesis is proposed that suppression of Numb, a Notch signal-suppressing molecule, results in promotion of Hes 1 transcription[32].

The small intestine from adult ICR mice was immunohistologically stained using anti-Musashi-1, Hes 1, and Ki-67 antibodies. Consequently, Musahi-1 was locally detected at the crypt base, but interestingly, also found to be strongly expressed immediately above Paneth cells where stem cells are concentrated as well as in CBC cells among Paneth cells. Hes 1 was similarly detected to be expressed immediately above Paneth cells and in CBC cells, but widely stained from the top of Paneth cells to the center of crypt, in contrast to Musashi-1. These Musashi-1 or Hes 1-positive cells were more strongly stained as compared to Ki-67-positive cells, indicating that they are proliferative cells. On the other hand, neither Musashi-1 nor Hes 1 was found to be expressed in absorbing epithelial cells, Paneth cells, and goblet cells (Fig. 2)[21].

Expression of Musashi-1 and Hes 1 was similarly studied in the postnatal development stage. On postnatal day 1 when the crypt was not yet formed,

Musashi-1 and Hes 1 were detected to be expressed in intervillous regions. On postnatal day 7, Musashi-1 and Hes 1 were both found to be expressed in the whole crypt, excluding Paneth cells. Furthermore, on postnatal day 14, 21, and so on, they were found to be localized as in adult animals [21].

In summary, Hes 1 was found to be positive widely in relatively undifferentiated cells at the villous origin and in CBC cells, but in contrast, Musashi-1 was stained only in CBC cells and cells immediately above Paneth cells, which were strongly positive for Ki-67 with potent proliferation. Namely, Musashi-1-positive cells were suggested to more specifically indicate stem cells. Furthermore, the number of Hes 1-positive cells definitely outweighed that of Musashi-1-positive cells, and therefore, it is not always correct to simply say that Hes 1 is present downstream Musash-1. On the other hand, Musashi-1 was strongly stained in conventional stem cells immediately above Paneth cells as well as in CBC cells, strongly suggesting that not only cells immediately above Paneth cells but also CBC cells constitute a part of stem cells. It has long been considered that CBC cells are not actual stem cells but progenitor cells specific for Paneth cells. Nonetheless, extremely long lifecycle of Paneth cells seems to be contradictory to potent proliferation of CBC cells, and as initially mentioned, it would be reasonable to consider that CBC cells are stem cells potential to be upward differentiated.

Epilogue : medical application of small intestinal stem cells

There are study reports on purification and isolation of stem cells from organs other than the small intestine, and a cell group with plasticity as well as ability of spontaneous proliferation was identified from the liver by a flow cytometry technique using surface marker [33]. However, most of stem cells isolated from the small intestine induced apoptosis while being isolated. Such experimental experiences suggest that microenvironments should be adjusted to maintain stem cells as they are. In future, it is considered necessary to elucidate and reconstitute microenvironments to maintain viable stem cells in order to utilize stem cells for regeneration medicine/therapy.

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