—**Review**—

Epithelial-Connective Tissue Cross-Talk Is Essential for Regeneration of Intestinal Epithelium

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Abstract

Epithelial cells of the gastrointestine undergo a rapid cell-renewal and originate from stem cells throughout the life of the organisms. Previous studies have provided a solid body of evidence to show that the epithelial cell-renewal is under the strict control of cell-cell and cell-extracellular matrix (ECM) interactions between the epithelium and the connective tissue. Especially, the microenvironment around the stem cells called "niche" is thought to play important roles in this control, and its disruption leads to diseases or disorders such as cancer in the human gastrointestine. Although understanding how the niche affects the stem cells is clinically important, its mechanisms still remain mostly unknown at the molecular level, possibly due to difficulties in the identification of the stem cells in the gastrointestine. Recent progress in cell and molecular biology is gradually beginning to shed light on some of the key signaling pathways in the cell-renewal of the intestinal epithelium, such as Wnt/T-cell factor (TCF) /β-catenin, Notch, Sonic hedgehog (Shh) /bone morphogenetic protein (BMP) signaling pathways, which are also involved in embryonic organogenesis and/or adult carcinogenesis. At present, only fragmentary information is available on their precise functions in the intestine. Nevertheless, there is a growing body of evidence that such signaling pathways have conservative functions in the intestine throughout terrestrial vertebrates, suggesting the usefulness of experimental animals to clarify molecular mechanisms regulating epithelial cell-renewal. In this article, I review some recent findings in this field, with particular focus on our studies using the *Xenopus laevis* intestine, where the stem cells form the mammalian-type intestinal epithelium under the control of connective tissue during metamorphosis. This *Xenopus* experimental system will certainly serve as a useful model for the study of the intestinal niche, whose clarification is urgently needed in regenerative medicine.
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Key words: intestine, tissue interaction, stem cell, regeneration, apoptosis

Introduction

All gastrointestinal epithelial cells originate from multipotent stem cells which reside in the special region of each organ throughout adulthood. In the small intestine, stem cells are localized near the bottom of the crypt, and their descendants actively proliferate and then, as they migrate along the crypt-villus axis, differentiate into absorptive cells,

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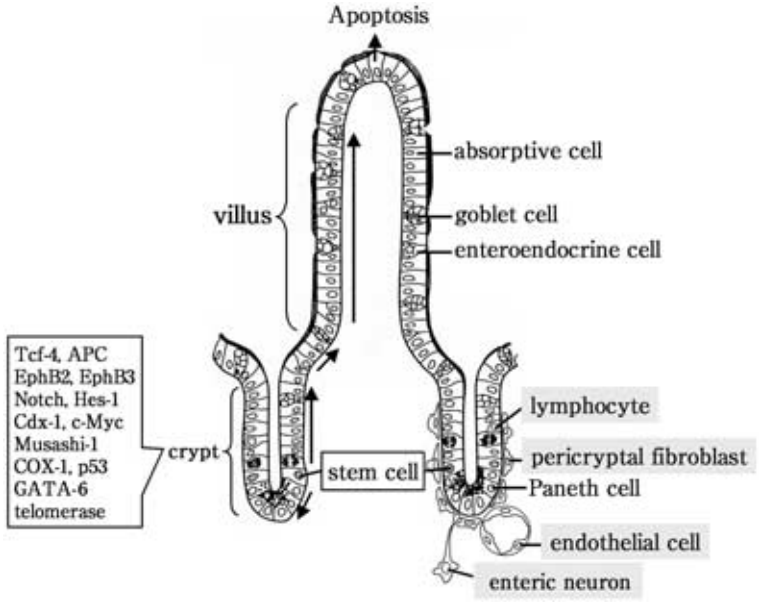

Fig. 1 Structure of the villus and the crypt in the mammalian small intestine. All of the epithelial cells originate from stem cells in the crypt, where many genes are expressed. A layer of pericryptal fibroblasts surrounds the epithelium of the crypt.

goblet cells, enteroendocrine cells, and Paneth cells[1]. Finally, differentiated epithelial cells degenerate through apoptosis. Thus, the homeostasis of the intestinal epithelium is based on the balance between cell proliferation, differentiation, and apoptosis. Loss of strict control over proliferation and/or apoptosis leads to cancer[2], and its mechanisms are clinically significant.

Previously, numerous studies pointed out that the microenvironment around the stem cells known as "niche" plays important roles in the control of epithelial homeostasis[3,4]. Morphologically, the epithelium of the mammalian intestinal crypt is surrounded by a layer of pericryptal fibroblasts, which migrate upwards with epithelial cells[5] (**Fig. 1**). In addition, a microvasculature consisting of endothelial cells[6] and intraepithelial lymphocytes[7] exist near the stem cells. These cells have been proposed as niche players involved in the maintenance and/or proliferation of stem cells and their descendants. More recently, enteric neurons that send their projections near the epithelium of the crypt have been shown to stimulate proliferation of progenitor cells committed to absorptive cells through enteroendocrine cell-produced glucagon-like peptide 2[8]. However, it remains mostly unknown what signal these candidate cells produce to affect the intestinal stem cells.

Genes Expressed in the Intestinal Crypt

Modern molecular biological techniques have gradually identified genes that are expressed in the mammalian small intestinal crypt (**Fig. 1**). Although only limited data are available at present about the functions of these genes in epithelial cell-renewal and their correlations with the cells described above, there are a few proposed key signaling pathways that are involved in epithelial cell-renewal. One is the Wnt/β-catenin signaling pathway, which plays important roles in the maintenance of stem cells. T-cell factor-4 (Tcf-4), the effector of its pathway, can be activated upon binding to β-catenin and up-regulates many target genes. Among them are (1) EphB2 and B3, which control cell migration in the crypt[9], (2) c-Myc, which generally stimulates cell proliferation[10], and (3) Cdx-1, which is associated with endodermal development during embryogenesis and has prooncogenic potential in the adult intestine[11]. Wnt protein activates this pathway by stimulating the formation of β-catenin-Tcf-4 complexes, whereas the adenomatous poliposis coli (APC) protein

competes with Tcf-4 to bind β-catenin and suppresses cell proliferation[12].

Another is the Notch signaling pathway, which is known in many other systems to affect cell fate decisions by lateral inhibition in cell-cell interactions through its receptor Delta. In the intestine, a high level of Notch is expressed in the stem cells and up-regulates Hes-1, a transcriptional repressor, which inhibits the expression of Math-1. Math-1 non-expressing cells remain in the progenitor pool and can only become absorptive cells, while Math-1 expressing cells differentiate into enteroendocrine, goblet, and Paneth cell lines[13]. Much remains to be learned about how these pathways interact with the niche players in the intestinal crypt.

Amphibian Intestine as a Model of Organ Regeneration

Amphibian metamorphosis bears many similarities to postembryonic organ development in mammals. In the *Xenopus* small intestine during metamorphosis, the larval epithelium undergoes apoptosis, whereas a small number of undifferentiated cells that express Musashi-1, a marker for mammalian intestinal stem cells and their immediate descendants, appear[14] (**Fig. 2A, B**) and form the adult epithelium. The adult epithelium is differentiated into all kinds of mammalian epithelial cells except for Paneth cells and acquires a cell-renewal system as the mammalian intestinal epithelium. Thus, the amphibian intestine provides a model for the study of intestinal epithelial regeneration.

It is well known that amphibian metamorphosis is triggered by a single hormone, thyroid hormone (TH). This means that molecular mechanisms of metamorphic changes can be clarified through the functional analysis of TH response genes. In the 1990s, a large number of TH response genes were isolated from the *Xenopus* intestine by using subtractive differential screening[15]. To assess their possible functions in the intestinal epithelium, we first examined the expression of TH response genes by *in situ* hybridization (ISH) and found some genes whose expression correlates well with the epithelial apoptosis or development of the adult intestinal epithelium.

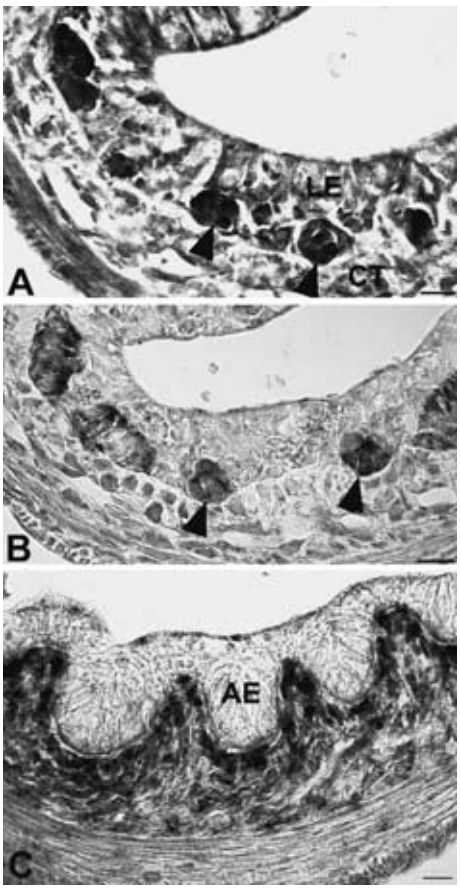

Fig. 2 Development of the adult intestinal epithelium during *Xenopus* metamorphosis. (A) Progenitor cells of the adult intestinal epithelium. They appear as islets stained strongly with methyl green-pyronin Y (**arrowheads**) between the degenerating larval epithelium (*LE*) and the connective tissue (*CT*). (B) Localization of Musashi-1 mRNA expression in the adult progenitor cells (**arrowheads**). (C) Fibroblast-specific expression of BMP-4 mRNA. When the adult epithelium (*AE*) grows in size by active cell proliferation, the level of BMP-4 mRNA becomes the highest with a gradient towards the epithelium. Bars, 20 μm.

(1) Roles of MMP11 in Epithelial Apoptosis through ECM

The larval epithelium undergoes massive apoptosis during a short period of metamorphosis.

Our previous electron microscopical study indicated that, concomitantly with the epithelial apoptosis, the basal lamina just beneath the epithelium suddenly becomes thick and permeable. This suggests that matrix metalloproteinases (MMPs), which are generally known to degrade various components of extracellular matrix (ECM), are involved in epithelial apoptosis. Among MMP genes such as collagenases, gelatinases, and stromelysins identified as TH response genes, stromelysin-3 (ST3; MMP11) gene is the only one whose expression in the connective tissue spatio-temporally correlates with apoptosis. In human tissues, the expression of MMP11 has been reported in both mesenchymal cells surrounding the epithelium undergoing apoptosis and fibroblasts surrounding invasive carcinomas[16]. However, its functions have not yet been experimentally demonstrated.

To clarify the functions of MMP11, we used the *Xenopus* culture system we established earlier, where both apoptosis and adult epithelial development can be induced by TH just like *in vivo*. The addition of function-blocking antibodies against the catalytic domain of MMP11 to the culture medium led to a dose-dependent inhibition of epithelial apoptosis as well as modification of the basal lamina. In addition, it also inhibited the invagination of adult epithelial cells into the connective tissue[17]. This study provided the first direct evidence of the effects of MMP11 on the epithelium. It is highly possible that MMP11 modifies components of the basement membrane ECM and causes signal transduction through epithelial cell surface molecules such as integrins, which results in epithelial apoptosis. How MMP11 regulates the expression of cell death genes such as caspases and cell death regulators remains to be determined.

(2) Shh/BMP Signaling Pathway Acting during Intestinal Epithelial Development

Another main process during intestinal epithelial regeneration is the development of the adult epithelium from the stem cells. We previously showed that fibroblasts that possess well-developed rough endoplasmic reticulum differentiate just beneath progenitor cells of the adult epithelium and exert inductive actions on adult epithelial development. Among TH response genes examined by ISH, the bone morphogenetic protein-4 (BMP-4), a member of the TGF-β superfamily, is noteworthy because its expression profile agrees well with the fibroblasts described above[18] (**Fig. 2C**). Furthermore, we have shown that Sonic hedgehog (Shh), whose epithelium-specific expression is directly up-regulated by TH, induces the expression of BMP-4 in fibroblasts. Recently, in higher vertebrates, the Shh/BMP signaling pathway is known to play important roles in the development of mesodermal tissues during gut organogenesis[19], although its roles in endodermal development are still not well defined. Also in the human gastrointestine, recent genomic data support the involvement of the Shh/BMP signaling pathway not only in embryonic organogenesis but also in homeostasis during adulthood. Mutations in different members of this pathway have been shown to be associated with malformations and diseases such as pre-cancerous juvenile polyposis syndrome (JPS)[20,21] (**Table 1**).

Table 1 Human gut malformations and diseases associated with the disruption of Shh/BMP signaling pathway

Signals	malformations and diseases
Shh	tracheo-esophageal (TE) fistula Anal atresia (AA) Intestinal metaplasia in the stomach
BMP	Juvenile polyposis syndrome (JPS) Hypertrophic pyloric stenosis (HPS) Hirschsprung diseases (HSCR)

We investigated the functions of Shh and BMP-4 in the intestine experimentally, using the *Xenopus* culture system. The addition of BMP-4 protein led to precocious differentiation of the intestinal epithelium, whereas its antagonist, Chordin inhibited its differentiation (unpublished data). On the other hand, excessive Shh protein caused anomalies of the intestinal epithelial structure and promoted cell proliferation in the connective tissue. These results strongly suggest key roles of Shh and BMP-4 in the development of the postembryonic intestine through

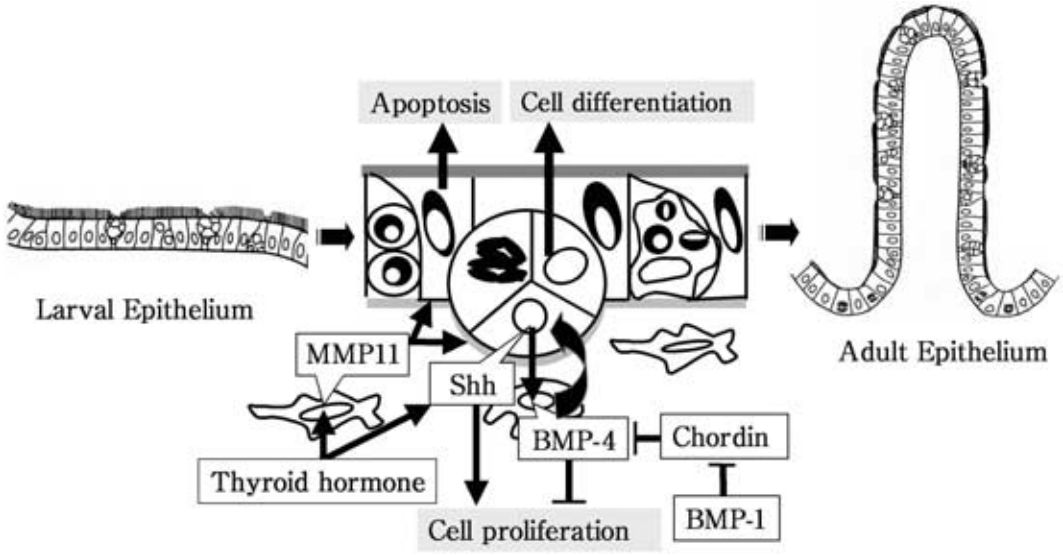

Fig. 3 Schematic drawing showing roles of TH response genes in intestinal epithelial regeneration during *Xenopus* metamorphosis. Thyroid hormone directly up-regulates the expression of MMP11 in fibroblasts and Shh in progenitor cells of the adult epithelium. MMP11 is involved in epithelial apoptosis through the modification of the basement membrane ECM. On the other hand, Shh induces the fibroblast-specific expression of BMP-4, which plays key roles in differentiation of the intestinal epithelium.

mutual epithelial-connective tissue interactions (**Fig. 3**). More precise functional analysis of this signaling pathway in intestinal regeneration is worth further study.

Conclusions and Prospects

The intestinal epithelium is continuously renewed from multipotent stem cells. The molecular mechanisms controlling the stem cells are clinically important and a full understanding of them is necessary for the therapeutic application of stem cells. To establish a common view of the intestinal stem cell niche, collaboration with investigators in multiple fields such as cell and molecular biology, pathology, and regenerative medicine are indispensable. Possibly, the most urgent task is to identify genes expressed in stem cells themselves and in their niche and to directly investigate their functions and mutual interactions. Since amphibian stem cells have many characteristics in common with their mammalian counterparts, and the genes regulating stem cells are easily identified as TH response genes, this animal provides a unique and excellent model in this field. Genes identified at present such as MMP11, Shh, and BMP-4 are the homologs of the corresponding mammalian genes and seem likely to have conserved functions throughout terrestrial vertebrates. In addition, the recently developed transgenic frog technology[22] will enable us to study gene function more directly and to gain clues to the mystery of the stem cell niche.

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