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How thyroid hormone regulates transformation of larval epithelial cells into adult stem cells in the amphibian intestine

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ABSTRACT

In the amphibian intestine during metamorphosis, a small number of larval epithelial cells dedifferentiate into adult stem cells that newly form the adult epithelium analogous to the mammalian counterpart, while most of them undergo apoptosis. Because this larval-to-adult intestinal remodeling can be experimentally induced by thyroid hormone (TH) both *in vivo* and *in vitro*, TH response genes identified in the *Xenopus* intestine provide us valuable clues to investigating how adult stem cells and their niche are formed during postembryonic development. Their expression and functional analyses by using the culture and recent transgenic (Tg) techniques have shed light on key signaling pathways essential for intestinal stem cell development. The present review focuses on such recent findings and discusses the evolutionally conserved roles of TH in development or maintenance of the stem cells which are common to the terrestrial vertebrate intestines.

1. Introduction

In the mammalian small intestine, adult stem cells are well known to be adequately controlled by the surrounding microenvironment called as the “niche”, which is required to maintain the rapid epithelial cell-renewal throughout adulthood. Although a growing body of evidence indicates important roles of the canonical Wnt and Notch signaling pathways in maintenance and proliferation of the adult stem cells (Clevers et al., 2014; Sato et al., 2011), the molecular mechanisms underlying formation of the stem cells and their niche still remains mostly unknown. The clarification of such mechanisms should be interesting from the viewpoint of stem cell and developmental biology and also be urgently needed for regenerative and cancer therapies.

During amphibian metamorphosis, most of the organs dramatically remodels from the larval aquatic to adult terrestrial form (Shi, 1999). In the *Xenopus laevis* small intestine, which is one of the best studied amphibian organs at the cellular level, larva-proper epithelial cells undergo apoptosis, while a small number of adult stem cells suddenly appear and newly form the adult epithelium possessing a cell-renewal system (Ishizuya-Oka and Ueda, 1996; McAvoy and Dixon, 1977) similar to the adult epithelium of the mammalian intestine (Cheng and Bjerknes, 1985; Madara and Trier, 1994). Since all processes of this larval-to-adult intestinal remodeling can be experimentally induced by simply adding thyroid hormone (TH) *in vitro* just as *in vivo* (Shi and Ishizuya-Oka, 1996), the amphibian intestine serves as a unique and invaluable model for the study of adult stem cell formation. From 1990s onward, a number of TH response genes have been identified in the *X. laevis* intestine (Buchholz et al., 2007; Das et al., 2009; Heimeier et al., 2010; Shi and Brown, 1993; Sun et al., 2013), and their expression and functional analyses now enable us to clarify the mechanisms of the intestinal remodeling. Here, I review the recent progress in this field, focusing on the roles of TH-activated signaling in the formation of the adult stem cells and their niche.

2. Amphibian intestinal model for adult stem cell development

2.1. TH-induced adult stem cells

The small intestine of *X. laevis* tadpoles before metamorphosis is a long and simple structure with only a single fold “typhlosole” (Marshall and Dixon, 1978a). Histologically, the tadpole intestine mainly consists of the simple columnar larval epithelium, immature connective tissue that is very thin except in the typhlosole, and

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thin layers of inner and outer muscles. The epithelial cells are differentiated as major absorptive cells possessing the brush border or secretory cells such as goblet cells. Until now, neither morphologically undifferentiated cells nor cells expressing any stem cell marker have been identified in the larval epithelium (Hourdry and Dauca, 1977; Ishizuya-Oka et al., 2003; Marshall and Dixon, 1978b). In the early period of metamorphic climax (around stage 60; Nieuwkoop and Faber, 1967), when the plasma TH level abruptly increases (Leloup and Buscaglia, 1977), the small intestine rapidly shortens. Most of the larval epithelial cells (larva-proper cells) begin to undergo apoptosis, whereas a small number of undifferentiated cells become detectable as small islets consisting of a single or few cells (Ishizuya-Oka and Ueda, 1996). These cells (adult stem/progenitor cells) are scattered in the entire epithelium between the larva-proper cells and the connective tissue, actively proliferate, and express a typical intestinal stem cell marker (Sun et al., 2010) the orphan leucine-rich repeat-containing G-protein-coupled receptor 5 (LGR5), Musashi-1 (Msi1) (Ishizuya-Oka et al., 2003), protein arginine methyltransferase-1 (PRMT1) (Matsuda and Shi, 2010), and sonic hedgehog (Shh) (Stolow and Shi, 1995), all of which are also expressed in crypts including stem cells and proliferating progenitors of the adult mammalian intestine (Kayahara et al., 2003; Nielsen et al., 2004; Sato et al., 2009). They rapidly grow into the developing connective tissue and then, as intestinal folds are formed, differentiate into the adult epithelium. By the end of metamorphosis (stage 66), the adult epithelium undergoes the cell-renewal along the trough-crest axis of the folds, similar to that along the crypt-villus axis of the adult mammalian intestine (Cheng and Bjerknes, 1985; Madara and Trier, 1994). That is, epithelial cells proliferate in the trough region of the folds and, as they migrate upwards, gradually differentiate, and finally undergo apoptosis at the tip of the folds. These similarities between the amphibian and the mammalian adult intestines enhance the usefulness of this animal model for the study of intestinal stem cell biology. In addition, we previously established an organ culture system and showed that TH can organ-autonomously induce the adult stem/progenitor cells in the *X. laevis* intestine at stage 57 (before metamorphic climax) after 5 days of TH treatment *in vitro* (Ishizuya-Oka et al., 2003). This indicates that the adult stem cells originate from the intestine itself but not from the other organs and that the molecular mechanism underlying formation of the adult stem cells should be clarified by assessing functions of TH response genes, which are endogenously expressed in the intestine (Shi and Ishizuya-Oka, 1996).

2.2. Epithelial origin of adult stem cells

To clarify whether the stem cells originate from the differentiated larval epithelium or not, we next performed tissue-recombinant culture experiments using wild-type (Wt) and transgenic (Tg) *X. laevis* tadpoles that constitutively expresses green fluorescent protein (GFP) (Ishizuya-Oka et al., 2009). The larval epithelium was separated from the other tissues (non-E) of the tadpole intestine at stage 57, recombined with homologous or heterologous Wt or Tg non-E, and cultured in the presence of TH. We showed that, whenever the epithelium is derived from the Tg intestine, regardless of whether it is recombined with Wt or Tg non-E, adult stem/progenitor cells express GFP. In contrast, whenever the epithelium is derived from the Wt intestine, they never express GFP. These results demonstrate that the stem cells originate from the epithelium alone. Because all of the larval epithelial cells before metamorphosis are at least partly differentiated and express no stem cell markers as mentioned above, it is concluded that some of the larval epithelial cells dedifferentiate into the adult stem cells. In accordance with this assumption, concomitantly with appearance of the stem cells, the expression of nuclear lamins changes from differentiated cell-specific lamin A to embryo-specific lamin LIII in such epithelial cells (Hasebe et al., 2011a), similar to the changes in the lamin expression during mammalian somatic cell reprogramming (Mitalipov et al., 2007; Miyamoto et al., 2007). Interestingly, in the mammalian intestine during maturation around birth, some fetal epithelial cells become the adult stem cells (Harper et al., 2011; Muncan et al., 2011), similar to the amphibian larval cells during metamorphosis (Ishizuya-Oka and Shi, 2011). Even in the adult mammalian intestine, partly differentiated epithelial cells such as transient amplifying cells (Potten et al., 1997) and secretory precursor cells (Visvader and Clevers, 2016) have been shown to retain the ability to dedifferentiate into the stem cells. Taken together, it seems likely that the intestinal epithelium, which is destined to be continuously renewed from the stem cells throughout the lifespan, generally pool such cells that can dedifferentiate into and function as the stem cells to prepare for emergencies.

2.3. Roles of tissue interactions in stem cell formation

Given that TH induces the larval epithelial cells to dedifferentiate into the adult stem cells, the next question arises whether TH directly acts on the epithelial cells or indirectly through the other tissues, or both. To address this question, we used Tg tadpoles that express a dominant positive TH receptor (dpTR) under the control of a heat shock-inducible promoter (Hasebe et al., 2011b). The dpTR functions as a constitutively liganded TR by specifically binding to the TH response elements of direct TH response genes and thus causes precocious metamorphosis even in the absence of TH (Buchholz et al., 2004, 2006). Tissue-recombinant experiments using stage 57-Wt and Tg tadpole intestines have shown that, whenever the epithelium does not express dpTR, no stem cell is detected in any recombinant intestine. In contrast, whenever the epithelium expresses dpTR, some of the epithelial cells express Shh, an early and direct TH response gene (Stolow and Shi, 1995), after 5 days of heat shock treatment *in vitro*. However, these cells do not fully dedifferentiate into the stem cells expressing markers such as Msi1, when they are recombined with Wt non-E. Only when the Tg epithelium is recombined with Tg non-E, that is, both the epithelium and non-E express dpTR, the stem cells are formed. These results led us to conclude that TH signaling in the surrounding non-epithelial tissues other than the epithelium is required for the formation of the stem cells, suggesting important roles of TH-inducible epithelial-connective tissue interactions during establishment of the stem cell niche.

In connection with this, we previously reported that the connective tissue rapidly develops by active cell proliferation and subsequent differentiation, concomitantly with adult epithelial development. Especially, remarkable ultrastructural changes occur in the epithelial-connective tissue interface around stage 60 (Ishizuya-Oka and Shimozawa, 1987). The thin basal lamina, a special extracellular matrix (ECM) separating the two tissues, suddenly becomes thick but amorphous and, through such modified basal lamina, fibroblasts possessing well-developed rough endoplasmic reticulum often make contacts only with the adult stem/progenitor cells. Thereafter, as the adult epithelium differentiates, its basal lamina becomes thin again, and the cell contacts become rare. This basal lamina modification following the cell contacts can be reproduced in the tadpole intestine *in vitro*, whenever the adult epithelium successfully develops (Ishizuya-Oka and Shimozawa, 1992). These observations imply that paracrine or juxtacrine signaling pathways between the epithelium and the connective tissue are involved in stem cell development.

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