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Drosophila as a Model for Intestinal Diseases

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Millions of people suffer diarrheal and inflammatory diseases of the intestine. Prolonged inflammation and tissue injury has also been proposed to potentiate gastrointestinal (GI) cancer. The GI tract encounters various ingested microbes and substances on a daily basis, in addition to the numerous commensal microorganisms that are already living inside. Pathogenesis can arise from an imbalance interaction between the host and these ingested microbes and chemicals. Drosophila has been a very useful model to study development and diseases. The relatively simple tissue organization, sophisticated genetic techniques and conservation of regulatory pathways are some advantages of using Drosophila as an experimental model. The local innate immune response in Drosophila GI tract involves reactive oxygen species and antimicrobial peptides. In addition, the pathogenic mechanisms of a few oral microbial pathogens for Drosophila are being investigated. Tissue damage in the intestinal epithelium is a common cause of pathogenesis. To understand how intestinal stem cells (ISC) mediate tissue repair during infection and disease progression can provide new therapeutic strategies. ISC in Drosophila midgut have been demonstrated to generate the various intestinal cell lineages. Moreover, genetic analyses have revealed the critical functions of Delta and Notch in ISC division and intestinal cell differentiation. Future studies using Drosophila as a model organism should provide important information regarding how pathogens cause tissue damage and stem cells mediate the repair in the intestine.

Key words: Drosophila, innate immunity, intestinal stem cell

INTESTINAL DISEASES

Around 1% of the US population experience inflammatory diseases of the intestine[1]. The death toll caused by diarrheal diseases is at 4 to 6 million per year globally[2]. Prolonged inflammation and tissue injury has also been proposed to potentiate gastrointestinal (GI) cancer[3,4]. The human GI tract is a complex organ. In addition to the digestive/absorptive functions, the GI tract is also an immune and endocrine organ, with immune cells and enteroendocrine cells surveying the gut and communicating with other organs[1,4]. Numerous commensal microorganisms are living inside the GI tract[5]. Moreover, the GI tract encounters ingested microbes and substances on a daily basis. Most of these microbes and ingested substances are beneficial or harmless. The health condition of the host, however, may cause a different reaction and the normally harmless substances may become pathogenic. To understand how cells in the GI tract interact with wide varieties of microbes and ingested substances is important for developing therapeutic strategies that alleviate intestinal diseases.

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DROSOPHILA AS AN EXPERIMENTAL MODEL

It has been well documented that fruit flies and humans employ conserved mechanisms in many biological processes. Some examples include homeodomain proteins in segmentation, Notch-Delta pathway in developmental cell fate switching and Toll-like receptors in innate immune response. Homeobox gene clusters were first identified in Drosophila and these genes are critical to define the segmental cell fate along the anterior-posterior axis. Evolutionarily conserved Hox gene clusters in mammals define the anterior to posterior development of axial skeleton, limbs and brain[6,7]. Delta and Notch are cell surface ligand and receptor, respectively. In Drosophila, the original functions of Delta and Notch are for switching between ectodermal and neural cell fates. Mammalian Delta and Notch functions are critical during T cell lineage switching, angiogenesis, and intestinal stem cell maintenance[8,9]. Droso-

phila Toll and mammalian Toll-like receptors have conserved functions in innate immune response. These receptors act to recognize, directly or indirectly, microbial compounds and in turn stimulate host defense response[10,11]. These conserved proteins not only control the development and health state of humans, the abnormal functions of these proteins are also linked to many diseases. Thus, results obtained from using Drosophila as an experimental model frequently provide important insights into normal physiological processes and disease progression in humans.

INFECTION AND IMMUNE RESPONSE IN DROSOPHILA GI TRACT

Drosophila does not have B and T cells and their host defense against invading microbes relies entirely on innate immune response[11]. An important part of systemic innate immune response is the increased production of antimicrobial peptides from fat bodies and the activation of hemocytes/blood cells (Figure 1). The antimicrobial peptides and hemocytes together suppress the initial titer of invading microbes. Other tissues such as epidermis and gut have barrier as well as active antimicrobial functions and they constitute the local innate immune response. Many aspects of innate immunity in Drosophila gut are being elucidated. Within the Drosophila gut, production of reactive oxygen species (ROS) is a critical mechanism for host defense against microbes. The production of antimicrobial peptides also plays a complementary role for host defense. These two protective mechanisms are critical for keeping the commensal and non-pathogenic bacteria in check[12-14]. Natural microbial pathogens through GI tract feeding, however, remain rare for Drosophila. Two known bacteria strains that can kill Drosophila by feeding are *Pseudomonas entomophila and Serratia marcescens* (*Db11*). Both strains can cause damage in gut epithelium and can evade to certain extent the innate immune function of the host[15,16]. However, the pathogenic and immune evasion mechanisms for P. e*ntomophila* and S. marcescens are still being investigated.

MAMMALIAN INTESTINAL STEM CELLS AND TISSUE REPAIR

When a host ingests chemical or microbial pathogens, a key problem is tissue damage along the GI tract. Thus, to understand how the tissue maintains its integrity by stem cell-mediated repair is important for developing future therapeutic strategies. By definition, intestinal stem cells (ISC) can self-renew and give rise to all mature cell types

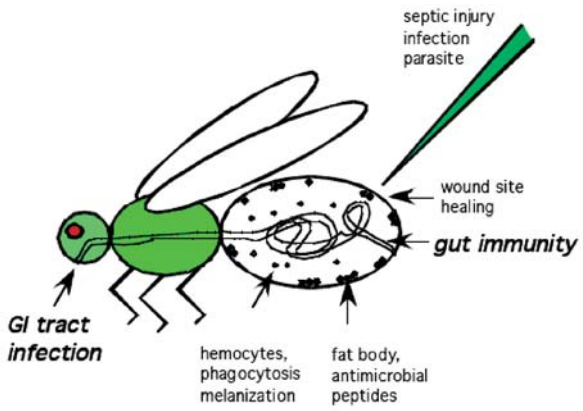

Fig. 1 Innate immune mechanisms in Drosophila. Infection by septic injury, oral feeding or parasite invasion can stimulate innate immune response in Drosophila. The various mechanisms of innate immunity includes antimicrobial peptides, hemocytes and wound healing. In addition, the local immune response in gut includes reactive oxygen species.

in the epithelium[17,18]. The ISC in mammalian small intestine is a well-studied system and is discussed here as an example to illustrate our current knowledge about ISC. Four main cell types are present in mammalian small intestine: enterocytes for absorption, globlet cells for mucus secretion, Paneth cells for antimicrobial peptide production, and enteroendocrine cells for hormone secretion[1,4]. ISC are located near the base of the crypts. ISC divide to give rise to transit-amplifying cells, which are precursor cells that can divide faster but each has committed to specific lineages. These precursor cells mature as they move up along the crypt-villus axis, replenishing the shedding epithelial cells at the tip of villus.

A major problem in ISC biology is that specific markers that can unambiguously identify ISC remain rare[1,4]. Previous experiments by long-term label retention suggest that ISC are located in the +4 region from the base of the crypt, referred to as +4 label retaining cells (LRC) (Fig. 1). These +4 LRC are relatively quiescent and can give rise to most cell types in the intestine. On the other hand, a recent report provides strong evidence that about 4-6 crypt-base columnar cells (CBC) located more proximal to the crypt base are ISC, as they express the specific marker Lgr5 and can give rise to all cell types after lineage labeling[19]. However, these Lgr5 positive ISC divide much faster than expected, once every 24 hours, because conventional wisdom argues that adult stem cells have limited capacity and should not be actively dividing. Although Lgr5 is a promising and highly

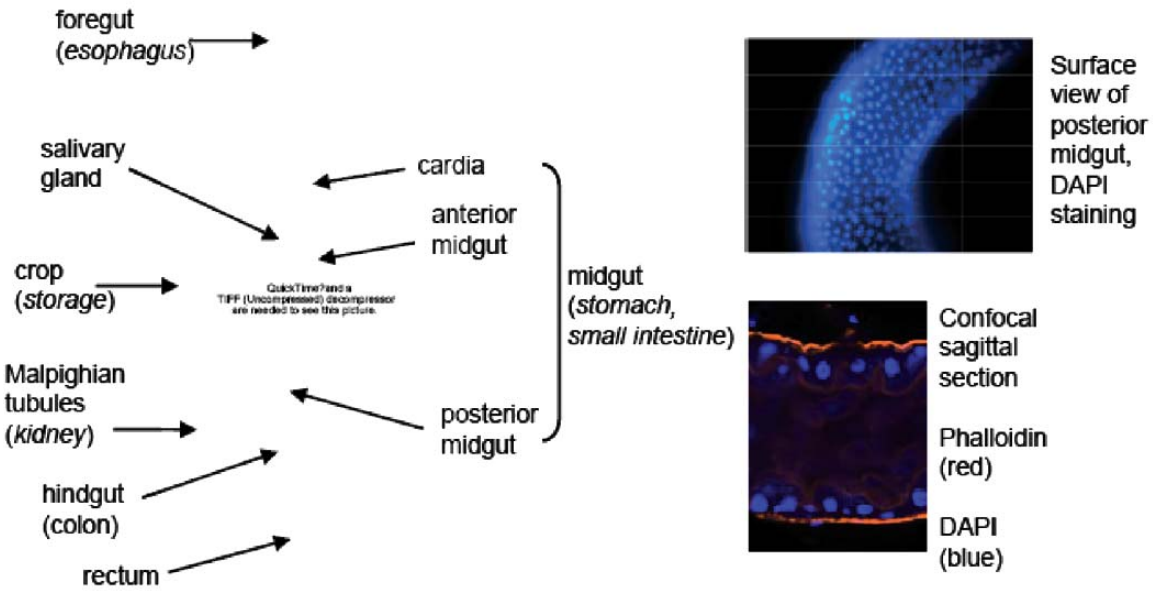

Fig. 2 Drosophila gut organization. The whole Drosophila gut from esophagus to rectum is presented and the various parts are labeled as indicated in the left panel. The counterparts in mammals are as indicated in parenthesis. The right panels are images of adult Drosophila midgut after staining with DAPI for DNA and phalloidin for actin. The small nuclei are ISC, enteroblasts and enteroendocrine cells, while the big nuclei are enterocytes.

specific ISC marker, the exact function of Lgr5 in ISC biology remains unknown. Further experiments are required to clarify the functional roles of +4 LRC versus Lgr5+ CBC as stem cells. Meanwhile, an inclusive model is that there may be both fast and slow dividing ISC in the intestinal crypt, and these two populations of ISC may be used under different circumstances or to replenish each other.

An assumption regarding tissue regeneration is that ISC can divide according to the need to replenish loss cells in the intestinal epithelium to achieve tissue homeostasis. However, in the mammalian intestine, relatively large number of fast dividing precursor cells are present in the transit-amplifying zone. Therefore, it has not been demonstrated clearly whether mammalian ISC can increase division in response to tissue injury. A previous report demonstrated that dextran sulfate sodium (DSS) caused injury in colon and increased proliferation of colonic progenitor cells[20]. In the absence of ISC specific markers, whether ISC were part of these proliferating cells was not clear. With the newly available specific marker Lgr5, similar experiments can be carried out again to examine the involvement of these CBC/ISC. Overall, many technical difficulties exist in examining how mammalian ISC respond to stimulation and initiate tissue repair. The use of model organisms particularly those that are amenable to genetic manipulation such as Drosophila may provide valuable insights into this research area.

DROSOPHILA INTESTINAL STEM CELLS

The GI tract of adult Drosophila is only approximately 1 cm long but contains cell types that resemble those in mammals. The Drosophila gut can be divided into 3 main portions: foregut, midgut and hindgut (Figure 2). The foregut includes mouth parts and esophagus. The midgut starts from cardia and extents to hindgut junction where malpighian tubules arise. The hindgut extends from the junction to anal plate. The three portions of Drosophila gut are formed independently but linked together during embryonic development. Thus, the origin of stem cells and cell fate determination may be different in these three portions.

Three recent reports demonstrate that ISC are present in adult Drosophila midgut[21-23]. The Drosophila midgut has a relatively simple organization and the structures are well formed at the time of hatching as an adult (Figure 2, right panels, big nuclei indicate enterocytes and small nuclei

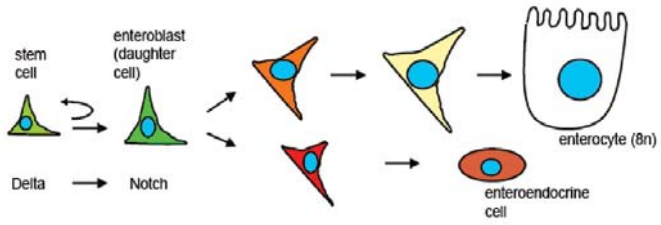

Fig. 3 Adult midgut ISC division and differentiation. ISC can give rise to all the cells in the two lineages in the midgut. When an ISC divide, it forms a self-renewed ISC and an enteroblast. The enteroblast is the precursor cell that can differentiate along either enterocyte lineage or enteroendocrine lineage. Delta and Notch signaling is critical for ISC identify and enteroblast differentiation.

indicate precursors and enteroendocrine cells). After hatching, the adult midgut does not have extensive growth or cell rearrangement. Thus, one extreme hypothesis was that the short life span of Drosophila allows fruit flies to go without ISC. Another hypothesis was that the adult midgut might not have ISC but instead precursor cells that had committed fate to replenish loss cells of specific lineages. Lineage tracing experiments reported in the last two years clearly demonstrate that in adult Drosophila midgut ISC are present to replenish all the cells types.

Two major mature cell types are present in Drosophila midgut: enterocytes (absorptive cells) and enteroendocrine cells (hormone producing cells) (Figure 3). Moreover, there are likely different subtypes of enterocytes, e.g. iron copper cells versus other enterocytes, and subtypes of enteroendocrine cells, e.g. Allatostatin versus Tachykinin expressing cells[22]. ISC and enteroblasts are the precursors. Within the midgut, the only cells that can go through mitosis are the ISC. The enteroblasts can have endo-replication during maturation process to become enterocytes, which are 8n polyploid cells. Because only ISC can go through mitosis, mitotic recombination technique was used to randomly mark ISC with GFP or lacZ expression for lineage tracing experiments[21,22]. By this lineage analysis technique, it has been demonstrated that ISC can give rise to both enterocytes and enteroendocrine cells.

CELL FATE DETERMINATION AND ISC SELF-RENEWAL

A key step in ISC self-renewal is to establish asymmetric fate between the two cells immediately after mitosis, that is renewed ISC versus enteroblast. Different stem cell systems in Drosophila use different strategies to establish asymmetry. One well-studied example is asymmetric localization of determinants such as Prospero before cell division in embryonic neuroblasts[24]. The second example is asymmetric usage of centrosomes to establish proper division axis in male germline stem cells[25]. In adult Drosophila midgut, the only known ISC-specific marker is Delta. Delta acts as an ISC membrane-bound ligand to stimulate the receptor Notch in the neighboring enteroblast (Figure 3). Although Delta functions as a cell surface ligand, the Delta protein needs to go through endosomal cycling in order to have activity, probably for the assembly of an active complex[26]. Thus, punctate cytoplasmic Delta is an indication of active Delta signaling. Only ISC exhibit punctate cytoplasmic Delta, thus serving as an ISC specific marker. When an ISC divide, Delta is segregated equally into both daughter cells. One daughter cell retains the cytoplasmic punctate Delta and becomes the renewed ISC, while the other daughter cell quickly loses the punctate Delta and becomes an enteroblast. The mechanism by which Delta is down-regulated in the newly formed enteroblast is not known.

Another proposed asymmetric cue is the extent of physical contact with the basement membrane, which lies between the basal side of the epithelium and the circular smooth muscle cells. ISC are in close contact with the basement membrane. When an ISC divides, the division angle between mitotic spindle and basement membrane is approximately 30°[23]. Therefore after division, one daughter cell remains close to the basement membrane, and this cell is the renewed ISC. The other daughter cell is farther away from the basement membrane and this cell becomes the enteroblast. A hypothetical model is that the basal localization of newly formed ISC activates a process that retains the active Delta, while the more apically localized enteroblast has less contact and cannot maintain the mechanism that retains active Delta. Once the asymmetric expression of Delta is established, the Delta in ISC acts as the ligand to stimulate the receptor Notch in the newly formed enteroblast. Notch signaling pathway in enteroblast is essential for the initiation of differentiation[21,22]. In loss of function Notch mutants, no differentiation of enteroblast is observed, and clusters of ISC-like cells are formed. It is obviously important to investigate the mechanism by which the division angle is determined. Equally interesting is to investigate whether the contact with basement membrane actually helps to maintain Delta expression and other stem cell properties of ISC. Many other interesting questions remain to be answered, such as how down-regulation of Delta occurs in the enteroblast, how ISC cell cycle is

regulated and whether ISC division can be regulated by different stimuli.

ENTEROBLAST DIFFERENTIATION PATHWAYS

Absorptive enterocytes and hormone secreting enteroendocrine cells are the two major mature cell types present in the midgut. Once an enteroblast is formed, the signals that govern the choice between these two differentiation pathways are almost completely unidentified. The only signal known is that high level of Delta in the original ISC correlates with an enterocyte fate, while low level of Delta in ISC correlates with an enteroendocrine fate[23]. Thus, the amount of Delta in ISC may determine the strength of Notch pathway within a newly formed enteroblast. The varying degree of Notch pathway activity may generate different outputs leading to the choice of different lineages. Furthermore, the same ISC can have varying levels of Delta. Thus, the same ISC can have daughter cells that can form either enterocytes or enteroendocrine cells. Approximately 90% of the enteroblasts will form enterocytes and 10% will form enteroendocrine cells[23]. Whether the level of Delta in ISC is a tightly regulated event or is an outcome of simple fluctuation is not known. The intermediate differentiation steps between enteroblast and mature cells are also not known.

CONCLUSION

The GI tract is a complex organ that is essential to the health of human beings. This organ encounters numerous stimulations on a daily basis. Entero-pathogens, both chemical and microbial, can cause cellular toxicity or stimulate excessive immune response. Tissue damage is an integral part of pathogenesis. Intestinal stem cells (ISC) play important roles in tissue homeostasis, by regenerating the various cell types in the intestine. The regulation of ISC division and cell type specific differentiation is not well understood but should be important for a better dissection of pathological conditions such as cancer progression and inflammatory bowel diseases. Drosophila midgut has a relatively simple cellular organization, and midgut ISC has recently been identified that functions to replenish the different cell types. By studying the mechanisms of ISC division in response to environmental challenges in the genetically amenable Drosophila system will likely provide important insights into similar processes in human GI tract.

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