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Review

Morphogenesis and maturation of the embryonic and postnatal intestine

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ABSTRACT

The intestine is a vital organ responsible for nutrient absorption, bile and waste excretion, and a major site of host immunity. In order to keep up with daily demands, the intestine has evolved a mechanism to expand the absorptive surface area by undergoing a morphogenetic process to generate finger-like units called villi. These villi house specialized cell types critical for both absorbing nutrients from food, and for protecting the host from commensal and pathogenic microbes present in the adult gut. In this review, we will discuss mechanisms that coordinate intestinal development, growth, and maturation of the small intestine, starting from the formation of the early gut tube, through villus morphogenesis and into early postnatal life when the intestine must adapt to the acquisition of nutrients through food intake, and to interactions with microbes.

Contents

1. Introduction

The mature intestine is a highly complex organ with several essential functions. The small intestine interacts with food after it has been digested in the stomach and broken down into simpler units. Carbohydrates, proteins, lipids, and other nutrients are absorbed by intestinal enterocytes and are absorbed into a highly integrated vascular network.

In addition to absorbing nutrients, the intestine has important roles in host immunity. Within the intestine, luminal contents come into contact with an epithelial layer, which must serve as a barrier to the outside environment and protect the body against indigenous (commensal) microbes and pathogens. Critical to this barrier are epithelial tight junctions which selectively limit the passage of luminal contents in between epithelial cells [1]. In addition, the epithelium secretes mucus, which lines the intestinal tract and serves as a dense barrier that can trap microbes to inhibit infection [1], and can also provide a rich source of nutrients for commensal bacteria [2,3]. Specialized cells of the intestinal epithelium also

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play an important role in host immunity by secreting antibacterial and antifungal peptides [4–6]. Moreover, colonization by commensal bacteria at birth stimulates immune system development and is necessary for proper immune function [7].

To adequately fulfill the cellular demands of these complex functions, the intestinal epithelium is organized into villi, which are finger-like structures that protrude into the lumen. The intestine has a high rate of epithelial cell turnover, driven by proliferative epithelial stem cells housed at the base of the villi in domains called crypts (Fig. 1). Stem cell driven proliferation fully regenerates the intestinal lining every 5–7 days [8–13]. As these stem cells divide, they differentiate and move along the villus structures like a conveyor belt. Once they reach the villus tips, cells undergo programmed cell death and slough off into the lumen.

The highly archetyped crypt-villus structures of the adult intestine emerge over developmental time through the coordination of several complex processes that govern tissue patterning, cell fate, and morphogenesis. Early in embryonic development, the intestinal epithelium is a relatively flat, tube-shaped structure which undergoes a process called villus morphogenesis through which the relatively flat tube-shaped intestine gives rise to villi and crypt structures. Villus structures project into the lumen, expanding the apical surface area of the absorptive epithelium. Morphogenesis of these projections is an evolutionarily conserved process, and therefore a positive adaptation of fitness, found in many vertebrates including the chicken and mouse, but also in vertebrates such as zebrafish, seahorses, snakes, and amphibians [14,15]. This morphogenesis is responsible for a massive expansion of intestinal surface area; it is estimated that the absorptive surface area of the adult human intestine is 30 m^2, with villus structures amplifying the surface area by 6.5 fold [16]. Abnormal loss of absorptive surface area hinders nutritional uptake and can lead to malabsorption or intestinal failure [17].

In this review, we will discuss the molecular, biochemical, and biophysical events that guide normal intestine development and functional maturation of the postnatal gut, with a focus on mammalian development including human intestinal development where possible. To further focus the review, we will cover developmental events starting after gut tube formation and through early postnatal life.

2. Intestine development

2.1. Models of the developing intestine

Historically, many studies of vertebrate intestinal development have been carried out in the chick and mouse. Chick embryos are easy to acquire, develop rapidly, are low cost, and can be easily manipulated experimentally. However, tools for genetic manipulation in a tissue specific manner are more limited in the chick. Additionally, there are significant differences between avian and mammalian gut development that may limit cross-species comparisons [18]. The embryonic mouse model develops in a similar time frame to the avian embryo (19-21 days), and as an advantage, has an extensive set of tools for tissue specific genetic manipulation. Drawbacks include larger housing costs, long breeding times to obtain genetic crosses, relatively small litter sizes, and internal development which hinders experimental access to the developing tissue. Most importantly, it is not entirely clear which aspects from these models may be directly applicable to human intestine development, since our understanding of human intestine development is severely limited at this time.

However, access to human fetal tissue and *in vitro* tissue culture techniques using human pluripotent stem cells (hPSCs) have begun to shed additional light into human intestine development. Indeed, recent access to high-resolution 3D-reconstructions of early stage human embryos in addition to histological sections will likely improve our understanding of the early stages of human fetal gut development [19]. However, most studies of human fetal tissue are limited to descriptive analyses. hPSCs, which include both embryonic and induced pluripotent stem cells, represent a highly tractable solution to the limitations inherent to fetal tissue. hPSCs can be differentiated into complex 3-dimensional (3D) intestinal tissue using soluble growth factors and/or small molecules in a step-wise process known as directed differentiation [20–22]. Directed differentiation aims to recapitulate key developmental stages *in vitro.* In the case of intestinal tissue, hPSCs undergo a gastrulation-like process that gives rise to a mixed endoderm/mesoderm population, followed by posterior patterning events, intestinal specification and gut-tube morphogenesis which gives rise to small self-assembling 3D structures that can be expanded into 'organoids' [23–26]. Intestinal organoids have been reviewed extensively elsewhere [22,27–31].

Recent studies have shown that intestinal organoids derived from hPSCs are most similar to fetal intestine [23,27,29,32] [33]. Intestinal organoids transplanted into the mouse kidney capsule engraft, form villus and crypt structures, and undergo enhanced cellular, molecular and structural maturation, resulting in more adult-like tissue [27,32]. In addition to hPSC-derived organoids, *in vitro* culture of primary human fetal intestinal epithelium (fetal organoids) is also shedding light on the cellular dynamics of the human fetal intestine [34]. Collectively, hPSC-derived organoids and fetal organoids provide a powerful new platform for investigating human development, since both systems are experimentally tractable, allowing for long- term growth, and genetic and pharmacologic manipulation.

2.2. Intestinal specification, gut tube patterning, and formation

In the case of human gastrulation, like the chick, the endoderm, mesoderm and ectoderm lineages are specified and are present as a flat, layered disc-shaped structure (reviewed elsewhere: [23,35–38]) As development progresses, the body of the embryo rotates from a flat to a fetal position where the ectoderm is present on the outside of the embryo and the endoderm, wrapped by mesoderm, is present on the inside of the embryo [39]. Conceptually, the endoderm can be visualized as a flat sheet of paper that is folded into a tube that must be sealed in the middle as the two sides come together. In the mouse, gut tube closure is complete by E9.0 [23,39], but mutant mice lacking Gata4, Sox17, and Furin/SPC1 fail to rotate properly and have open gut tubes [40–44].

During embryo rotation and coinciding with complex morphological events that shape the tissue, the nascent gut tube is patterned into different domains along the anterior-posterior axis. Secreted morphogens help to establish region-specific gene regulatory networks, segmenting the gut tube into domains with distinct molecular characteristics that will ultimately give rise to different organs [45–47]. This process is reviewed in detail elsewhere [20,22,23,35,39,48–52]. For example, the foregut and hindgut domains of the endoderm are separated by expression of Sox2 and Cdx2, respectively [48,49,53,54]. The anterior region of the gut tube, which gives rise to the esophagus and stomach in addition to the lungs, liver, and pancreas, initially expresses Sox2, which sets up a sharp boundary at the pylorus [48,55]. Adjacent to this Sox2 boundary is the posterior region of the gut tube, which will give rise to the small and large intestine, marked by Cdx1, 2, and 4 expression [35,48,54,56–60].

Interestingly, while Cdx (Cdx1, 2, 4) proteins have been shown to play redundant roles in intestinal patterning during development [61–63], Cdx2 is considered to be a master regulator of intestinal identity; conditional deletion of Cdx2 in the epithelium