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Review

Epithelial stem cells and intestinal cancer

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ABSTRACT

The mammalian intestine is comprised of an epithelial layer that serves multiple functions in order to maintain digestive activity as well as intestinal homeostasis. This epithelial layer contains highly proliferative stem cells which facilitate its characteristic rapid regeneration. How these stem cells contribute to tissue repair and normal homeostasis are actively studied, and while we have a greater understanding of the molecular mechanisms and cellular locations that underlie stem cell regulation in this tissue, much still remains undiscovered. This review describes epithelial stem cells in both intestinal and non-intestinal tissues, as well as the strategies that have been used to further characterize the cells. Through a discussion of the current understanding of intestinal self-renewal and tissue regeneration in response to injury, we focus on how dysregulation of critical signaling pathways results in potentially oncogenic aberrations, and highlight issues that should be addressed in order for effective intestinal cancer therapies to be devised.

1. Introduction – the adult multifunctional epithelium

The mammalian adult epithelium is an essential layer of cells that lines the cavity and lumen of multiple organs in the body. It is found as part of a membranous barrier or as part of ducts and secretory glands. Epithelium can be either monolayered or multilayered (stratified), and contain both proliferative and differentiated cell types that perform its highly specialized functions. The adult epithelium is truly multifunctional – it can protect, secrete and absorb, often performing all these activities within a single organ. In skin, for instance, the stratified epithelium maintains a protective barrier between the body and its surroundings, and so protects underlying tissues from physical traumas and environmental stresses, while its secretory function is mediated by glands that release sweat [1]. In the gastrointestinal tract, the monolayered epithelium is constantly bombarded by physical and biochemical insults. Thus, not only does it function as a barrier to ingested pathogens, the gastrointestinal epithelium simultaneously secretes gastric acids, mucous, proteases and hormones for digestion of food. It also controls other gastric processes (*e.g.* motility) and nutrient and water uptake through differentiated absorptive enterocytes of the villi.

Epithelial cell repair and regeneration in these tissues is thought to be driven by dedicated stores of stem or progenitor cells found in defined niches. These stem cells generate and replace damaged epithelia throughout the lifetime of the animal. For epithelia of the skin and gastrointestinal tract, which are subjected to substantial injury, epithelial cell turnover occurs rapidly to maintain constant tissue repair and regeneration [2]. Indeed, the gastrointestinal epithelium represents one of the organs with the fastest cellular turnover, with a daily loss of 10 [11] epithelial cells in humans [3,4]. In other epithelia, such as those of the lungs, active epithelial regeneration occurs only in response to injury [5].

In the last decade, much has been discovered about the existence and characterization of stem or progenitor cells in epithelial tissue systems comprising the skin, hair follicle, mammary gland, stomach and gastrointestinal tract [6–11]. Of these, the gastrointestinal stem cell field has probably made the most progress, in terms of understanding the locations and molecular pathways that regulate stem cell activity and behavior. In this review, we explore the regenerative and stem-cell driven nature of the epithelium in relevant tissue systems during normal cellular homeostasis, with a focus on the gastrointestinal epithelia, and how dsyregulation of certain molecular pathways in these cells contributes to intestinal tumourigenesis.

2. Epithelial stem cells in non-intestinal tissues

The LGR proteins (LGR4, LGR5 and LGR6) are 7-transmembrane G-protein-coupled receptors that function as Wnt coreceptors. LGR

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proteins bind their secreted ligands, the R-SPONDINS, to potentiate Wnt signals and regulate downstream signaling pathways. They have recently emerged as one of the more predominant markers that define epithelial stem cells. Indeed, epithelial stem cells in tissues exhibiting a constant turnover are often characterized by their enriched expression of at least one Lgr protein [12,13].

2.1. Skin and hair follicle

The hair follicle in the skin is composed of a bulge linked by an isthmus portion of the hair shaft, the sebaceous gland and the interfollicular epidermal layer. Epithelial stem cells of the hair follicle express all three Lgr proteins, albeit in different regions. *Lgr4* expression is broadly observed from the bulge up to the isthmus, in a pattern that somewhat overlaps the expression of *Lgr5* and *Lgr6*. *Lgr5+* stem cells are found exclusively in the bulge and are responsible for generating all mature follicle lineages, except the sebaceous gland, which is instead produced by *Lgr6+* cells. These are detected predominantly in the intermediate isthmus portion, and can also produce the interfollicular epidermis. Regeneration of both the sebaceous gland and the interfollicular epidermal layer by *Lgr6+* stem cells has been demonstrated by *in vivo* lineage tracing as well as transplantation experiments, thus proving that *Lgr6+* cells are a major adult stem cell reservoir for this organ [11].

2.2. Stomach

The adult stomach is segregated into an upper portion (the corpus), and a lower portion adjacent to the small intestine (the pyloric antrum) [12]. Both parts are lined by an epithelial layer containing multi-lineage gastric gland units that invaginate into the mucosal layer of the stomach. The multiple lineages that make up these gland units originate from epithelial stem cells that reside at the base of the glands [14]. Originally, the presence of this multipotent stem cell region was demonstrated by inhibition of ubiquitous *LacZ* expression within the gastric epithelium of adult *Rosa26LacZ* mice. The resulting observation that all four main gastric lineages were detected in *LacZ*-negative clones indicated that these mature cells came from a single common multipotent stem cell. These experiments were random in nature however, and the identity of the multipotent stem cells could not be proven until much later when Barker et al. [2,6] applied a more targeted lineage tracing method (see Section 3.1) on the intestine and stomach. In these experiments, activation of *lacZ* within gland-base *Lgr5*-expressing cells demonstrated that these cells could supply all the epithelial lineages of the gastric gland. Importantly at later time points, these glands appeared to have become dominantly populated by cells derived from a single *Lgr5+* clone, suggesting that these *Lgr5+* epithelial stem cells drove regeneration in the stomach. Recently, gastric corpus cells which expressed the tumor necrosis factor (TNF) and stem cell marker *Troy* were also found to be enriched for *Lgr5*, and were capable of replenishing whole gastric units [15]. As for other Lgr proteins, adult stomach epithelial stem cells do not express *Lgr6*, and the expression of *Lgr4* is as yet undocumented.

Together, these tissues illustrate the role of specialized epithelial stem cells in the renewal of critical organs, and further underscore the importance of the Lgr proteins in maintaining their stem cell function. A more comprehensive discussion of these non-intestinal systems can be found in Barker et al. [16], Vries et al. [12], and Barker et al. [13].

3. Epithelial stem cells in the gastrointestinal tract

The mammalian gastrointestinal tract is a long series of tubular organs that can be functionally and morphologically separated into two main parts – the small intestine and the large intestine or colon [13,16,17]. Although the various constituent organs are distinct in terms of function and gene expression, their basic anatomical architecture is similar. Muscular peristalsis pushes digesting food through the tract and across an inner surface of connective and supportive stromal tissues (*i.e.* lamina propria) and intestinal mucosa. This mucosa is an absorptive epithelium, and yet between highly related tissues such as the small intestine and colon, its biological functions and tertiary architecture are noticeably different. Derived from the endoderm, the intestinal mucosa is of great interest in the study of gut regeneration and disease pathogenesis, as it contains defined niches with multipotent stem cells that facilitate and mediate many cellular processes (*i.e.* self-renewal, fate determination and differentiation, proliferation and apoptosis).

3.1. Early gastrointestinal tract development

Following gastrulation in mammals, the definitive endoderm (DE) germ layer is induced to form by signaling from the TGFß ligand, Nodal [18–21]. In the mouse, the DE layer at both the anterior and posterior tips of the embryo fuses shut from E7.5-9.5, and invaginates towards the center of the embryo to establish the primitive gut tube [17,22] (Fig. 1). From E9.5, the pseudostratified DE develops along the interior surface of the primitive gut, and continues to thicken until E14.5, when extensive remodeling converts the DE into a monolayered epithelium lining a tubal structure [22]. Further elongation and patterning of the tubal structure defines the fore-, mid- and hindgut, with the hindgut eventually giving rise to the gastrointestinal tract.

3.2. The postnatal gastrointestinal tract: structure and homeostasis

From late embryogenesis to early postnatal development, the epithelium of the small intestine arranges itself into finger-like projections known as villi, which reach into the lumen of the intestine and serve to absorb nutrients. At the base of these villi are the crypts of Liekerkühn ('crypts') – epithelial pockets or invaginations that constitute instructive niches for small reserves of multipotent cells (Fig. 2). Soon after the crypts form [23], increased stem/progenitor cell activity results in a greater quantity of epithelial cells [24]. Thus, by the end of the third postnatal week when the intestine tackles its first solid meal, the intestinal epithelia is fully equipped with the capability to renew and regenerate itself. Intestinal crypt cells give rise to transit-amplifying (TA) cells, which in turn undergo many cycles of rapid proliferation and progressively commit to one of several differentiated lineages as they migrate upwards along the crypt–villus axis [25]. Amongst these functional lineages, two predominant specialized epithelial lineages emerge–absorptive and secretory cells (Fig. 2, right). Absorptive enterocytes secrete enzymes and assimilate nutrients, while secretory cells include hormone-secreting enteroendocrine cells, mucous-secreting goblet cells and prostanoid-secreting Tuft cells. Paneth cells, which help to maintain intestinal stem cell (ISC) reserves and also have antimicrobial functions, and the antigen-sampling Microfold cells, are also found in the crypts. After 3–5 days, migrating epithelial cells reach the villus tip, where they undergo apoptosis before being sloughed off into the intestinal lumen [26–29]. Paneth cells escape this rapid ascension to certain death, instead migrating downwards toward the crypt base [30] where they remain for 6–8 weeks before being replaced by local progenitor populations [13,31]. The epithelial lining of the colon, however, does not contain absorptive villi and Paneth cells. This makes it much flatter on the luminal side and reflects its role in stool compaction rather than food absorption. The need to efficiently expel stool is reflected by the presence of many more mucous-secreting goblet cells in the colon. In the small intestine, about 250 new cells are generated and replaced