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Review article

Gastrointestinal organoids: How they gut it out

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ABSTRACT

The gastrointestinal tract is characterized by a self-renewing epithelium fueled by adult stem cells residing at the bottom of the intestinal crypt and gastric glands. Their activity and proliferation is strongly dependent on complex signaling pathways involving other crypt/gland cells as well as surrounding stromal cells. In recent years organoids are becoming increasingly popular as a new and powerful tool to study developmental or other biological processes. Organoids retain morphological and molecular patterns of the tissue they are derived from, are self-organizing, relatively simple to handle and accessible to genetic engineering. This review focuses on the developmental processes and signaling molecules involved in epithelial homeostasis and how a profound knowledge of these mechanisms allowed the establishment of a three dimensional organoid culture derived from adult gastrointestinal stem cells.

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Contents

1. Introduction 1
2. Molecular mechanisms in the gastrointestinal tract 2
    2.1. Intestine 2
    2.2. Stomach 4
3. Translating knowledge into the dish 4
    3.1. Intestinal Organoids. 4
    3.2. Stomach organoids 8
    3.3. Gastrointestinal cancer organoids. 8
4. Concluding remarks and future perspectives 8
Acknowledgments. 9
References 9

1. Introduction

The gastrointestinal tract is a continuous structure and the digestive part can be divided into the stomach, the small intestine and the large intestine. These organs are in constant contact with nutrients, metabolites and resident bacteria, which are on the one hand indispensable for human health, but on the other hand inflict constant damage including genetic alterations to the epithelium. Consequently, the gastrointestinal tract requires continuous self-renewal to maintain its epithelium and minimize the accumulation of otherwise cancerogenous mutations. This is achieved by a population of adult stem cells (AdSC) residing in spatially restricted parts of the respective tissue. There they continuously divide and give rise to progenitor cells further dividing and differentiating into every cell type present in the respective tissue. In the intestine, the turnover rate for an entire renewal is about 3–5 days (Darwich et al., 2014). This fast renewal and the related cell division activity of the adult stem cells are orchestrated by a complex network of signaling pathways involving different cell populations. Deregulation of the fine-tuned balance between activation and suppression of these signaling pathways results in

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malignant transformation (Barker et al., 2007), making it a medical necessity to understand the processes regulating stem cell homeostasis. The impact of malignant transformation of gastrointestinal stem cells is evident in cancer statistics: stomach cancer and colorectal cancer are the 3rd and 4th most frequent reason for cancer associated death worldwide, accounting each for around 700.000 victims per year (Globocan, 2012).

Several decades of research and recent cutting edge findings have revealed the developmental processes and molecular mechanisms of tissue homeostasis taking place in the intestinal crypt and gastric gland. In this context it is noteworthy that numerous findings originated from comparisons with cancer development. Tumors as well as stem cells are influenced by their surrounding niche. The clarification of the fundamental mechanisms active in the intestinal stem cell niche allowed the reproduction of this niche in a 3D *in vitro* culture system. Under selected growth conditions stem cells proliferate and form complex structures called organoids, which have been developed for the intestine (Sato et al., 2009) as well as for the stomach (Barker et al., 2010). Organoids represent an ideal tool to study developmental processes and tissue maintenance mechanisms due to their ease of genetic manipulation. The technique has spread widely within the community, mainly because of the potential of organoids to model *in vitro* many complex *in vivo* processes. In addition, although being somatic cells, they can be cultivated far beyond the Hayflick limit (Hayflick, 1965) while maintaining their genomic stability (Huch et al., 2015).

Here we present an overview of developmental processes and signaling pathways active in the mammalian gastrointestinal tract and how they translate into the establishment of organoid cultures. We discuss state of the art culture conditions as well as future challenges and opportunities in the field of gastrointestinal organoids.

2. Molecular mechanisms in the gastrointestinal tract

2.1. Intestine

The intestinal epithelium is composed of units formed by villi and crypts. In the embryonic intestinal tract proliferation is taking place in small invaginations developing into crypts (Patzelt, 1882; Pfitzner, 1880), which then give rise to villi and other crypts (Paneth, 1887). In the adult intestine cellular division is only occurring in the crypt but not in the villus (Bizzozero, 1893). The renewal of an entire unit is performed by a conveyor belt mechanism from the bottom of the crypt to the top of the villus driven by self-renewing, undifferentiated and multipotent stem cells (Friedman, 1945; Leblond and Messier, 1958; Leblond and Stevens, 1948; Quastler and Sherman, 1959; Stevens and Leblond, 1947; Walker and Leblond, 1958). Several potential stem cell populations have been proposed in the crypt (Behnke and Moe, 1964; Hampton, 1968; Kataoka, 1970; Troughton and Trier, 1969). One of them named crypt based columnar (CBC) cells is closely associated with Paneth cells at crypt bottoms (Cheng and Leblond, 1974). CBCs together with Paneth cells have long been proposed to form a restricted stem cell zone within the crypt (Bjerknes and Cheng, 1981a, 1981b, 1981c), a postulation that has been confirmed experimentally by lineage tracing experiments (Barker et al., 2007). Confined zones of proliferative and more differentiated regions can also be observed in tumors (Vermeulen and Snippert, 2014). Signaling pathways relevant in tumors such as e.g. the Wnt or Notch pathway often also play a decisive role in the stem cells of the respective tissue. In most colorectal cancers (CRCs) deregulation of genes associated with the Wnt pathway is an initiating event and results in enhanced proliferation (Groden et al., 1991; Kinzler et al., 1991; Korinek et al., 1997; Liu et al., 2000; Rubinfeld et al., 1993; Su et al., 1993). Interestingly, CRCs have overlapping gene expression patterns with intestinal crypts (van de Wetering et al., 2002), e.g. the surface protein Lgr5. Lineage tracing experiments revealed that single Lgr5$^+$ CBC cells are able to regenerate an entire crypt-villus axis. These cells are in a state of “stemness” and possess long-term self-renewal capabilities as well as multipotent differentiation abilities (Barker et al., 2007), thus fulfilling the definition of a stem cell (Lajtha, 1979). The size of the intestinal stem cell compartment remains constant over time. Cell population size is essential in this setting and involves self-renewing as well as differentiated cells. The cellular divisions follow a stochastic model with the stem cells dividing symmetrically upon the loss of a nearby cell, a concept known as neutral drift dynamics. In this stochastic model single intestinal crypts inevitably drift into monoclonality (Snippert et al., 2010). Several tissues contain Lgr5 positive stem cells e.g. the hair-follicles (Jaks et al., 2008) or the gastric antrum (Barker et al., 2010) making it a general marker of stemness in several Wnt-dependent tissues. Nevertheless, Lgr5 expression by itself does not convey stemness per se, as some differentiated cells can express Lgr5, hinting to the importance of additional factors such as the surrounding stromal niche in the transmission of stemness.

Research in the past years has revealed a complex signaling network present in the intestinal crypt. CBC stem cell activity is maintained by continuous communication with the surrounding Paneth cells and surrounding stromal cells. Several signaling mechanisms are involved, among them Wnt, BMP/TGF-β, Notch and EGF with Wnt signaling being a key regulator of epithelial homeostasis and self-renewal activity (He et al., 2004; Schuijers et al., 2015). While the cells move along the crypt-villus axis they are exposed to a Wnt gradient. Stem cells become loaded with Wnt mediators that are produced by adjacent Paneth cells, which bind to their cognate Frizzled receptors (Farin et al., 2016). Due to the local production and limited diffusion, Wnt molecules as well as their receptors are diminished through turnover by cellular division as the cells leave the stem cell zone and move away from Paneth cells. Besides Lgr5 the CBC stem cells express a whole set of further Wnt pathway associated genes like e.g. the transcription factor Ascl2 (van der Flier et al., 2009a, 2009b), which directly controls stemness in the intestinal crypts (Schuijers et al., 2015). The high Wnt activity in CBC stem cells is mediated by binding of secreted Rspondin family members to Lgr family members on the CBC membrane (Carmon et al., 2011; Glinka et al., 2011; de Lau et al., 2011). This binding potentiates the Frizzled mediated Wnt pathway activation. The molecular mechanism underlying this potentiation are two ubiquitin ligases, Rnf43 and Znrf3 (Koo et al., 2012), which mediate the endolysosomal degradation of Frizzled receptors by ubiquitination for fine regulation of Wnt activity in the intestinal stem cell. Upon binding of Rspondin to a Lgr receptor, Rnf43 and Znrf3 are recruited to a tripartite complex with Rspondin and Lgr5, sequestering them from Frizzled. As a result, robust activation of the Wnt pathway is achieved by blocking the inhibitory action of Rnf43 and Znrf3 (Hao et al., 2012). Another cell type at the crypt bottom relying on Wnt pathway activity is the Paneth cell. The downstream Wnt-target gene *Sox9* is important in the development of Paneth cells (Bastide et al., 2007; Mori-Akiyama et al., 2007) as well as maturation of secretory precursor cells into Paneth cells (Andreu et al., 2005, 2008; van Es et al., 2005a). As already mentioned, Paneth cells also play an important role in stem cell niche formation at the crypt bottom (Fig. 1A). Besides this, Paneth cells are a major source of intestinal antimicrobial proteins in the form of lysozyme and defensins, protecting the crypts and thus the stem cells from microbial assaults. A defective defensin production followed by a break-down of the mucosal antibacterial defense is a critical pathogenetic factor in

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