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THE SMALL INTESTINAL MICROBIOME: VIBING WITH INTESTINAL STEM CELLS

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Abstract

The epithelial lining of the small intestine mediates its absorptive and secretory function and thus is a critical component of human health. Regeneration and renewal of the epithelium is the result of proliferation of intestinal stem cells (ISCs). Many cell types and molecular factors are known to regulate the ability of ISCs to proliferate, including adjacent neighboring epithelial cells and the underlying, supportive stromal cells. The microbiome resides in the lumen of the small intestine and is in close contact with the epithelium. Due to its proximity to ISCs, it has been hypothesized that species within the microbiome have the capacity to regulate ISC proliferation and differentiation. This review highlights research that probes interactions between ISCs and the microbiome in the small intestine to detail the current understanding of microbial regulation of ISCs. Results from these studies provide important knowledge that can be exploited to identify therapeutic targets or develop novel preventative treatments to treat intestinal diseases.

Introduction:

The small intestinal epithelium is a single layer of diverse types of secretory and absorptive cells that mediate intestinal function. The epithelium is divided into two microdomains or compartments: the villus and the crypt. The crypt region is characterized by invaginating pocket-like structures that are surrounded by the underlying stroma, while the villus region protrudes into the intestinal lumen to increase surface area for nutrient absorption. The crypt is home to intestinal stem cells (ISCs), that undergo two types of cell division. Symmetric division generates new ISCs for self-renewal, and asymmetric division gives rise to daughter cells that migrate from the crypt region into the villus region, where they undergo differentiation to functional secretory and absorptive cells. ISCs continuously proliferate and differentiate, replacing the mature cells that line the villus region every three to five days. The microenvironment of the small intestinal crypt where ISCs reside is termed the “ISC niche.” Components of the ISC niche include the surrounding differentiated epithelial cells that remain in the crypt instead of migrating to the villus, and the underlying stromal cells, a heterogenous group of cells that reside in the connective tissue including

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mesenchymal, immune, neuronal, and vascular cells. The epithelial and stromal components of the ISC niche serve as a well-known, major source of signals that modulate ISC proliferation and differentiation (Santos et al., 2018, Greicius and Virshup, 2019). Recently, the human microbiome has emerged as another source of factors that regulate the function of ISCs. The advent of the Human Microbiome Project resulted in a wealth of information regarding the putative microbial composition in the small intestine (Human Microbiome Project, 2012), and a recent focus on the functionality of these organisms has resulted in the discovery that the microbiome and ISCs have an intimate relationship (Peck et al., 2017). This review will discuss the regulation of ISCs, the current understanding of the landscape of the small intestinal microbiome and its products, and the evidence that the microbiome is a source of signals for ISCs and thereby a major component of the ISC niche. It is important to comprehend how ISCs interact with the small intestinal microbiota and are influenced by bacterial products, as this knowledge enhances our understanding of the factors that assist in maintaining epithelial homeostasis and contribute to renewal and repair in the context of small intestinal damage.

Regulation of the Intestinal Stem Cell:

ISCs in the small intestine undergo continuous rounds of cell division approximately every 24 hours (Potten, 1998). As they divide in the base of the crypt, undifferentiated daughter cells migrate upwards through the transit-amplifying (TA) zone near the crypt-villus axis, where they continue to divide as they progress upwards towards the villus. As the cells move up the villus, they differentiate into their respective functional cell types, including enteroendocrine cells, which secrete hormones, enterocytes, which absorb nutrients, tuft cells, which perform chemosensory functions, and goblet cells, which secrete mucus. The exception to this upwards movement of differentiating cells are Paneth cells, which reside in the base of the crypts, where they secrete anti-microbial factors and shape the intestinal microbial composition (Figure 1) (Clevers, 2013, van der Flier and Clevers, 2009, Barker et al., 2012, Tian et al., 2011, Bevins and Salzman, 2011, Metcalfe et al., 2014, Clevers and Bevins, 2013). Apart from Paneth cells and a few enteroendocrine and tuft cells that remain in the crypt region, differentiated daughter cells migrate to the tip of the villus, where they are eventually shed into the small intestinal lumen by a process called anoikus. As ISCs are primarily responsible for this process of generating new daughter cells that differentiate into the mature intestinal cell types, regulation of ISCs is tightly controlled to ensure normal epithelial homeostasis and renewal, and conversely, is dysregulated in many small intestinal diseases.

Many markers have been used to identify ISCs, facilitating the study of ISC regulation. Currently in the small intestine, the most well-studied crypt ISC marker is a cell surface receptor named LGR5 (leucine-rich-repeat-containing G-protein-coupled receptor 5, also known as Gpr49). Other markers that have been associated with ISCs include OLFM4, KI67, PCNA, SOX9, ASCL2, and AXIN2 (Barker et al., 2012, Burclaff et al., 2022). Postulated quiescent or reserved stem cells – also called +4 ISCs – reside directly above the terminal Paneth cells in the beginning of the TA zone, and are marked by BMI1, HOPX, TERT, and LRIG1 (Burclaff et al., 2022, Duckworth, 2021, Yan et al., 2012). The existence of quiescent ISCs is controversial, and unlike the actively dividing LGR5+ ISCs,

quiescent ISCs are not thought to be the primary source of epithelial renewal in homeostatic conditions, but are rather believed to play a major role in regenerative repair in the context of LGR5+ ISC damage, especially following radiation exposure (Andersson-Rolf et al., 2017, Metcalfe et al., 2014, Dheer and Young, 2021). Control of LGR5+ ISC proliferation and differentiation in the small intestine has been linked to conserved signaling molecules and pathways, including wingless/integrated (WNT), R-spondin, epidermal growth factor (EGF), Hedgehog, Notch, Gremlin, and bone morphogenetic proteins (BMPs) (Vanuytsel et al., 2013). Key factors in these pathways are provided to ISCs by various cells that comprise the ISC niche (Duckworth, 2021, Greicius and Virshup, 2019, Santos et al., 2018), namely Paneth cells, which reside in between the actively dividing LGR5+ ISCs (Figure 1) and provide WNT and Notch ligands to ISCs (Clevers and Bevins, 2013), and stromal cells, such as telocytes and myofibroblasts, which reside underneath the epithelium and serve as a source of WNTs, R-spondin proteins, and EGF (Greicius et al., 2018, Greicius and Virshup, 2019, Gregorieff et al., 2005, Kabiri et al., 2014). The canonical WNT pathway is the most well-established regulator of ISCs (Barker et al., 2008, Barker et al., 2007, Barker et al., 2012, Logan and Nusse, 2004, Katoh, 2007, Katoh and Katoh, 2007), and WNT proteins secreted from stromal or Paneth cells locally activate intracellular signaling cascades within neighboring ISCs in a paracrine fashion (Sato et al., 2011, Greicius and Virshup, 2019, Greicius et al., 2018, Kabiri et al., 2014, Katoh, 2007, Katoh and Katoh, 2007, Theodosiou and Tabin, 2003). WNT target genes play an indispensable role in maintaining stemness and promoting proliferation in the small intestine, and WNT signaling thereby serves as the master regulator of proliferation in the ISC niche (Logan and Nusse, 2004, Katoh and Katoh, 2007, Biechele and Moon, 2008). In addition to their role in small intestinal homeostasis, many of these proliferation-promoting factors, such as WNT, have been shown to play a vital role in ISC recovery after damage (Gehart and Clevers, 2019, Santos et al., 2018, Greicius and Virshup, 2019, Duckworth, 2021).

The Microbiome:

The microbiome consists of more than 300 trillion bacteria, fungi, and viruses (Dave et al., 2012, Human Microbiome Project, 2012) that colonize the gastrointestinal tract. The small intestinal organisms are dynamic due to the changing conditions in the small intestine caused by intact of food, the intermittent secretion of enzymes and factors, and short transit time(Kastl et al., 2020). The composition of organisms varies by region with increasing amount and diversity from the proximal to distal portions (Kastl et al., 2020). Interactions between these organisms and ISCs could occur through two possible points of contact: direct communication between the organism and ISCs or communication with a niche cell that then indirectly signals ISCs (Peck et al., 2017, Savage and Blumershine, 1974, Nelson and Mata, 1970). Although tantalizing data suggests small intestinal microbes play a role in regulating ISCs, the underlying mechanisms that form the basis for microbiome-ISC interactions have not been elucidated. Filling this knowledge gap has been challenging, partly due to the lack of *in vitro* models in which microbe-epithelium interactions can be interrogated, the diversity of the gut microbiome and factors it produces, and the difficulty in pinpointing exactly what organisms are present in the small intestine. Most of the studies investigating small intestinal microbe-ISC interactions have utilized murine

models. Although much has been learned from these studies, the composition of the murine microbiome is distinct from humans due to many factors, including host genetics, differences in the composition of outer intestinal mucosa layer that affects mucus-associated bacteria survival, lower intestinal pH values, and differences in diet (Arrieta et al., 2016, Hugenholtz and de Vos, 2018). Humans and mice share only 15% of intestinal bacteria lineages, and while murine models can provide valuable insights into ISC-microbiome interactions, the many differences between the human and mouse intestinal microbiomes highlight the need to study these interactions in a complimentary human model system (Arrieta et al., 2016). Human intestinal organoids provide a new model system in which human microbe-ISC interactions can be interrogated *ex vivo*, as they enable the culturing of human ISCs, model differentiation of the human epithelium, and facilitate the exploration of microbial-ISC interactions in a reductionist environment (Blutt et al., 2018, Foulke-Abel et al., 2014, Zachos et al., 2016). New advances in human organoid cultures will be addressed below.

The Landscape of the Intestinal Microbiome and Its Metabolites:

The composition of the small intestinal microbiome is highly variable based on the genetics of the individual and environmental factors such as age, diet, and antibiotic use (Hasan and Yang, 2019, Kurilshikov et al., 2021). The microbiome produces many factors that appear to modulate its functional role in the intestine, including short chain fatty acids (Boffa et al., 1992, Ichikawa and Sakata, 1997, Lee et al., 2017, Lee et al., 2018), tryptophan metabolites (Roager and Licht, 2018), polyamines, (Wang et al., 1991), secondary bile acids (Kozoni et al., 2000, Pai et al., 2004), vitamins (Lai et al., 2021), reactive oxygen species (Morris and Jasper, 2021), and hydrogen sulfide (Xing et al., 2020). Most of the research characterizing the overall intestinal microbiome has utilized 16S gene content analysis in stool, which is easily collected via non-invasive methods (Dave et al., 2012, Tang et al., 2020, Human Microbiome Project, 2012) but reveals very little about what might be present in the small intestine. Recent studies have revealed differences between the composition of the stool microbiome compared to the microbiome found at the intestinal epithelial surface (Vuik et al., 2019, Vasapolli et al., 2019). Differences in bacterial composition also exist between the luminal microbiota and the microbiota proximal to the epithelium, potentially because epithelial-associated organisms have unique properties allowing them to utilize nutrients and adhere to glycans within the mucous layer that coats the epithelial surface (Robbe et al., 2004, Juge, 2012). The composition of the microbiome also varies by region in the intestine and increases in diversity from the small intestine to the large intestine, with a smaller bacterial load and less-diverse microbiota associated within the upper duodenal regions of the small intestine and the largest numbers and greatest diversity of bacteria found in the terminal large intestine (Martinez-Guryn et al., 2019, Zhang et al., 2014). The diversity of the bacterial communities along the different regions of the intestine also contributes to the spectrum of products produced by microbes in each intestinal region, thereby affecting the local interactions of microbes and their metabolites with the epithelium (Wang et al., 2005, Stearns et al., 2011).

While researchers are making strides in gaining a deeper understanding of which bacteria inhabit the upper and lower regions of the human gastrointestinal tract, the composition

of the human microbiota that colonizes the middle portions of the small intestine – the jejunum and upper ileum – has remained elusive (Dave et al., 2012, Tang et al., 2020, Kastl et al., 2020). Difficulties in assessing the microbiota in this region is mainly a result of technical challenges in sampling these regions through traditional endoscopy and colonoscopy methods, as the middle regions of the small intestine are not easily reached by scope equipment. Additionally, utilizing animal models to make these discoveries is limited by the substantial variations in microbial composition between animals and humans. Advancements in identifying which bacteria reside in the small intestine may be achieved by examining small intestinal tissues obtained through organ donation. By scraping the intestinal tissue, organ donor samples can be used to isolate microbes found in these regions from healthy individuals (Sartor, 2015).

Role of the Microbiome on Intestinal Stem Cell Regulation:

The idea that small intestinal microbiome plays a role in modulating ISC activity results from its proximity to the epithelium and ability to secrete factors that can modulate ISC biology. Indirect evidence for microbial regulation of ISCs originated from observations by Gordon and Bruckner-Kardoss in 1961 that germ-free mice showed reduced intestinal surface area compared to that of conventionally raised mice (Gordon and Bruckner-Kardoss, 1961). Many other studies have observed the phenomena of reduced villus height, crypt depth, and mitotic indices in the small intestine of germ-free or antibiotic-treated mice when compared to mice with a unaltered microbiome (Lesher et al., 1964, Greig et al., 2018, Khoury et al., 1969), suggesting that the microbiome can control epithelial regeneration and renewal. Further support for a link between the microbiome and ISCs comes from reports that colonization with microbes restores normal intestinal epithelial histology in germ-free mice, rats, and *Drosophila* (Gordon and Bruckner-Kardoss, 1961, Buchon et al., 2009a, Banasaz et al., 2002). Evidence for direct interactions between microbes and ISCs has also emerged from studies by Lee *et al.* where microbially produced lactate was shown to enhance proliferation in the murine small intestine via the stimulation of the LGR5 receptor on ISCs (Lee et al., 2018). It remains to be seen whether this activation of LGR5 ISCs occurs because microbially derived lactate interacts directly with ISCs or indirectly via the stimulation of other cell types of the ISC niche (Table 1). Although an in-depth mechanism of how the microbiome regulates ISCs remains to be fully elucidated, evidence from the literature suggests that a complex network of microbes and their metabolites are clearly involved in regulating ISC activity through various niche pathways, in the context of both homeostasis and damage.

Many species and strains of intestinal bacteria have been implicated in affecting ISC proliferation and differentiation (Table 1). Due to the amount of genetic variability between strains of a bacterial species – especially in those that were isolated from different hosts (Frese et al., 2011) – it is probable that many of the observed effects of a given microbe are strain-specific. For example, in mice, *Lactobacillus reuteri* strain 17938 increases murine small intestinal crypt cell proliferation, but strain 6475 does not (Preidis et al., 2012). Comparative genomics reveals that although these strains are members of the same species, they share only 70% of their genes (Saulnier et al., 2011). As these strains differ in their ability to induce crypt cell proliferation, these genetic differences may code for factors

that modulate ISCs, potentially providing key targets for further exploring the relationship between ISCs and the microbiome. Strain-specific differences are particularly relevant when drawing conclusions from studies linking specific microbial metabolites or factors from a microbial species to ISC regulation, as not all strains of a species may produce the same factor.

Many different mechanisms have been proposed to explain how microbes regulate ISCs (Table 1 and Table 2), suggesting that microbial regulation of ISCs is most likely complex and multi-factorial, involving multiple pathways and mechanisms. Of the major niche pathways, the mechanism often proposed for microbial stimulation of ISC proliferation is the activation of WNT/β-catenin signaling (Kim et al., 2021, Wu et al., 2020, Lyu et al., 2022), which is unsurprising, as this pathway is the major proliferation-stimulating pathway in LGR5+ ISCs, and without proper WNT/β-catenin signaling, the intestinal epithelium is unable to self-renew (Korinek et al., 1998, Pinto et al., 2003, Kuhnert et al., 2004). Whether microbes induce these pathways directly, with microbial cells themselves interacting with the epithelial surface, or indirectly, via microbial metabolites, secreted proteins, or outer membrane vesicles remains poorly understood. Due to the presence of the mucus barrier that overlays the intestinal epithelium in the absence of injury or disease, many studies have concluded that microbes are unable to physically interact with the epithelium, hypothesizing that the ISC niche is a sterile environment, free from microbes themselves (Hansson and Johansson, 2010, Johansson et al., 2008, Johansson et al., 2014). However, the crypt location would be ideal for microbial modulation of ISC pathways through direct microbial communication with the various ISC niche components—potentially through interaction of microbial cell wall components with the epithelium or through the release of outer membrane vesicles or metabolites that can locally interact with the niche—yet whether these direct interactions occur remains unknown.

In addition to WNT/β-catenin signaling, other pathways of microbial regulation of ISCs have also been suggested. ISCs robustly express the pattern recognition receptor Nod2, which recognizes the peptidoglycan motif muramyl dipeptide (MDP) that is a cell wall component in all bacteria (Nigro et al., 2014, Ogura et al., 2003). This finding serves as a clue that microbial cells may be able to physically interact directly with ISCs. MDP has been linked to beneficial effects in epithelial repair and has recently emerged as a microbial-responsive mechanism that aids in ISC survival in murine and organoid studies. Nod2 has been shown to trigger a pathway of ISC cytoprotection when stimulated by MDP; a mechanism thought to enhance the ability of ISCs to regenerate crypts upon exposure to cytotoxic stressors such as reactive oxygen species, radiation damage, or the chemotherapeutic agent doxorubicin (Nigro et al., 2014, Levy et al., 2020, Lee et al., 2019).

Many microbially derived metabolites and secreted products are thought to provide important components to the ISC niche (Table 2). Recognition that small intestinal microbial products such as MDP and lactate can maintain the proliferative capacity of the ISC niche in the context of damage highlights the importance of maintaining a diverse, healthy microbiome. Several strains promote proliferation and facilitate epithelial renewal following damage, as seen in DSS-induced mouse colitis models, radiation-induced rat injury models, and during pathogenic infection (Wu et al., 2020, Zhang et al., 2020, Zhang et al., 2023,

Lu et al., 2020, Hua et al., 2023) (Table 1). These findings demonstrate that microbes play an important role in repairing the small intestinal epithelium following damage. Commensal microbes, such as *Lactobacillus reuteri* D8, can stimulate epithelial proliferation and repair to reduce intestinal pro-inflammatory cytokine secretion and serum LPS concentrations (Wu et al., 2020). Additionally, in the context of damage due to inflammation or radiation injury, commensal microbes – especially lactobacilli – have been documented to play a very important role in facilitating small intestinal epithelial renewal by stimulating ISC proliferation via IL-22 secretion from immune cells (Zhang et al., 2023, Hua et al., 2023, Qiu et al., 2017, Ge et al., 2022, Hamade et al., 2022, Hou et al., 2018). In contrast, other evidence suggests that certain microbes might enhance damage. The microbial endotoxin lipopolysaccharide (LPS), which is often present in higher amounts during pathogenic infection, has been shown to inhibit cellular proliferation and increase apoptosis in bot the small intestine upon binding to the toll-like receptor 4 (TLR4) (Naito et al., 2017, Neal et al., 2012). Further work is necessary to understand both the positive and detrimental effects of the small intestinal microbial composition on ISC regulation.

Ways to Study Microbial Regulation of Intestinal Stem Cells:

Most of the research studying the pro-proliferative effects of various microbes on the ISC has been conducted in murine models. However, it has not yet been ascertained how applicable these discoveries in murine systems are to human biology. The expanded use of human intestinal organoid model systems provides the opportunity to overcome the hurdles of host and species-specific differences. In 2011, Sato *et al*. pioneered the development of *ex vivo* tissue-derived human small intestinal organoids (HIOs), which allow the direct cultivation of human ISCs *in vitro* (Sato et al., 2011). Unlike transformed cell lines, HIOs are genetically stable and closely model the cellular makeup of the *in vivo* intestinal epithelium (Blutt et al., 2018, Foulke-Abel et al., 2014, Sato et al., 2011, Sato et al., 2009), which presents many advantages. HIOs can be grown in multiple formats, facilitating the study of many scientific questions involving microbe-epithelial interactions (Figure 2). In a 3D format, bacteria can be microinjected into the lumen of the HIO to assess how live microbes affect the rate of organoid growth and ISC proliferation (Poletti et al., 2021). Williamson *et al*. recently developed a high-throughput method of 3D organoid microinjection to evaluate how various microbes influenced gastrointestinal physiology (Williamson et al., 2018). Additional studies in 3D HIOs have analyzed the impacts of microbes or their secreted products on various aspects of epithelial biology (Co et al., 2019, Dheer and Young, 2021). HIOs can also be grown in a 2D monolayer or transwell format, which allows easy access to the epithelial apical surface for the application of microbes or their products and more closely mimics the physiologic contact of the epithelium and the intestinal microbiome (VanDussen et al., 2015, Wilke et al., 2020). Transwells also facilitate access to the basolateral side of the epithelium to address microbial-cell interactions that may occur in this region and model systemic infections (Wang et al., 2018). Work in organoid models has allowed researchers to determine the colonization patterns of microbes and how microbial factors affect the apical surface of the epithelial cells (Rajan et al., 2018, Zhang et al., 2020). As HIOs have the advantage of preserving the host's genetic makeup *in vitro*, microbial-HIO studies can encompass multiple individuals with a variety of genetic

backgrounds, permitting the assessment of demographic factors such as sex, ethnicity, and age to be studied in the context of the microbiome. The advent of HIOs presents a powerful tool that can be used to gain a comprehensive understanding of the intricate interplay between the human small intestinal microbiome and ISCs. To circumvent disparities in the microbiota between mice and humans, future research endeavors should supplement rodent models with the integration of microbiome components into HIOs.

Additional methods to study complex communities of bacteria are being developed that, when combined with organoid technology, can be used to assess host-microbe and ISC-microbe interactions. Robert Britton's laboratory pioneered the development of mini-bioreactor arrays (MBRAs) that can be utilized to cultivate complex human small intestinal microbial communities and metabolites (Auchtung et al., 2015). Using the MBRA system, stable microbial communities can be reliably and reproducibly cultured within eight days, with approximately 94% of the microbes present from the original sample represented in the MBRA community (Auchtung et al., 2015). Supernatants from these communities can be collected and their biological effects on ISCs assessed using several different assays. Other laboratories are actively expanding the repertoire of culturable bacteria; an advancement that, when paired with HIO studies, will substantially contribute to the scientific community's ability to uncover the mechanistic relationship between complex communities of microbes and ISCs (Wang et al., 2018, Rettedal et al., 2014, Kim et al., 2016, Kim et al., 2022, Afrizal et al., 2022).

Microbial Regulation of the Large Intestine Stem Cell:

Unlike the small intestinal epithelial landscape, which has a crypt and villus architecture, the colonic epithelial landscape is much different, and only consists of deep glandular crypt regions. The colon is home to the greatest and most diverse population of microbes in the body; therefore, it is also important to understand how the resident bacteria in the colon may be impacting the regeneration processes of the colonic stem cell. Due to the ease of sampling and characterizing the microbiome in the large intestine, there is a plethora of research examining whether large intestinal microbial communities modulate ISCs. Driving this work is the association of colorectal cancer and the microbial composition in the large intestine. Large intestinal microbial dysbiosis strongly correlates with the development of cancer in the large intestine(Kim and Lee, 2021) and there is much focus on understanding the effect of the large intestinal microbiome on epithelial proliferation.

Several groups have found that various large intestinal microbes and microbial products can influence ISC proliferation in the colon including *Bacteroides fragilis, and Lactobacillus rhamnosus* GG (Wu et al., 2003, Zhang et al., 2023, Darby et al., 2020). Colonic organisms have been postulated to interact with the colonic ISC via several different mechanisms, including a toxin-mediated destruction of epithelial E-cadherin which subsequently triggers an epithelial repair response characterized by β-catenin translocation to the nucleus, a hallmark of ISC activation(Wu et al., 2003). Other mechanisms include activation of STAT2 signaling via the induction of IL-22 (Zhang et al., 2023), leptin mediated induction of epithelial proliferation, and proliferation via pathways that involve Nox (Darby et al., 2020). Most of the work linking specific large intestinal microbial metabolites to ISC regulation

involve short-chain fatty acids (SCFAs), such as butyrate, acetate, propionate, and valproic acid, which are produced by microbial fermentation of dietary polysaccharides and are found primarily in the colon. The most well-studied SCFA is butyrate, which serves as a direct energy source for colonocytes and has been shown to modulate inflammation (Lee et al., 2017, Donohoe et al., 2011, Dou et al., 2020, Chang et al., 2014). Butyrate can stimulate proliferation in healthy colonic epithelium but inhibits proliferation in tumor cell lines (Whitehead et al., 1986, Sakata, 1987, Kien et al., 2007, Frankel et al., 1994), a phenomenon known as the “butyrate paradox” (Mariadason et al., 2001, Comalada et al., 2006). Although detailed mechanisms explaining how colonic microbial metabolites regulate ISCs have not been fully elucidated, evidence from the literature suggests that many microbial metabolites can regulate epithelial proliferation. With the high prevalence of colonic cancers worldwide (Boustany et al., 2023), it is important to understand the relationship between resident colonic microbiota and their influences on epithelial proliferation. Recent work has identified a crypt-specific microbiota that resides deep within murine colonic crypts (Pedron et al., 2012, Saffarian et al., 2019) which implies local interactions between the two entities. Further research is needed to fully understand and appreciate whether organisms that live in the large intestinal crypt region provide local signals that regulate ISCs.

Conclusion and Discussion:

The intestinal lumen is a highly dynamic and changing environment that is home to trillions of bacteria that constitute the intestinal microbiome, a complex ecosystem of organisms that live in symbiosis with the intestinal epithelium. Epithelial maintenance is critical for intestinal health and originates from ISCs. The relationship between the intestinal microbiota and ISCs is highly dynamic and understanding its complexity is still in its infancy. Rodent, zebrafish, and *Drosophila* model systems provide solid evidence that the microbiome plays an important regulatory role in ISC functioning. In addition to functioning under homeostasis, the microbiome has also been linked to enhanced intestinal repair mechanisms that occur following injury. A deep focus on the role of the microbiome in regeneration and renewal following intestinal damage will begin to shed light on the utilization and manipulation of the microbiome to treat intestinal disease. Collectively, this area of research implies that the intestinal microbiome holds tremendous potential as a therapeutic target. In addition, the microbiome can be regulated by many external and internal factors including dietary factors (Perler et al., 2023) such as fiber (Myhrstad et al., 2020) and sugars (Di Rienzi and Britton, 2020), prebiotic (Bedu-Ferrari et al., 2022), probiotic (Hemarajata and Versalovic, 2013) and antibiotic use (Fishbein et al., 2023), stress (Segerberg-Konttinen, 1988), physical activity (Holzhausen et al., 2022), sleep (Klimashina et al., 1989), aging (Badal et al., 2020), genetics (Hall et al., 2017), and disease (Durack and Lynch, 2019). Understanding how these variables indirectly affect ISC dynamics and whether they have the potential to modulate intestinal regeneration and repair via their effects on the microbiome is an emerging area of research. It is tantalizing to speculate as to whether simple interventions such a modulation of diet might modulate ISC renewal in humans, as this has been demonstrated in mice (Hou et al., 2021). Deeper knowledge of microbiome-ISC interactions will provide an understanding of the potential application of the microbiome to human health. We predict that the field will continue to advance with the

use of new model systems to fully understand how the microbiome “vibes” with ISCs in humans and further define the human ISC niche.

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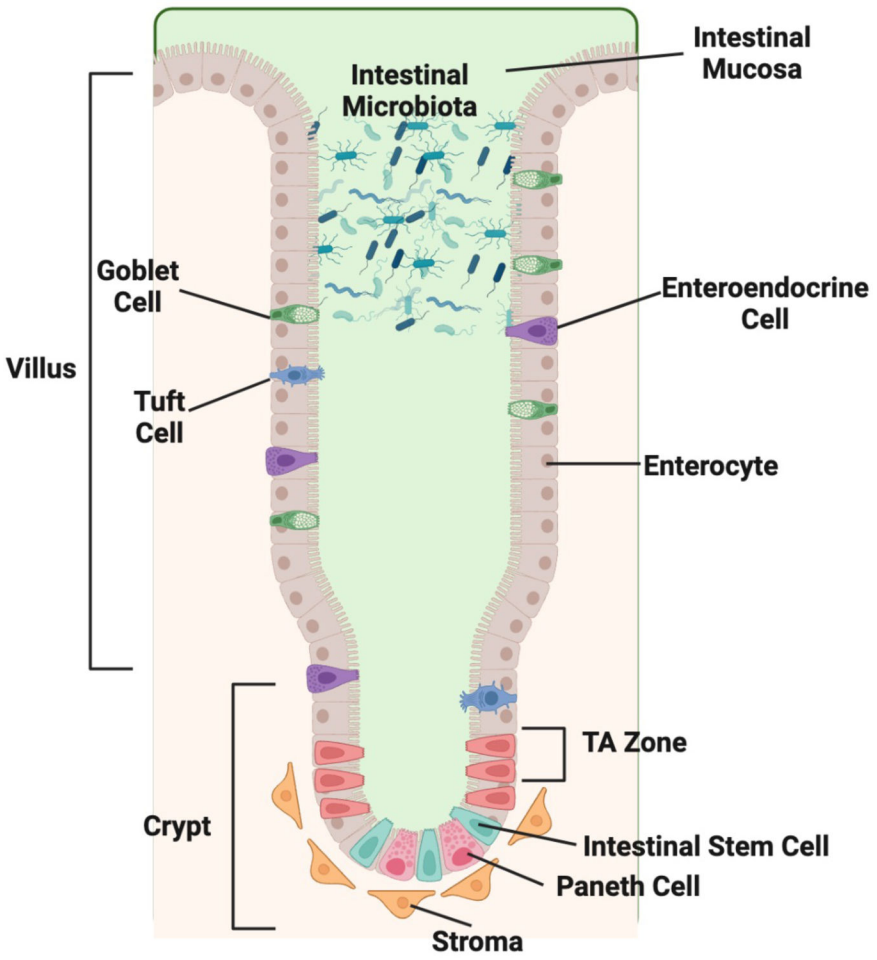

Figure 1: Landscape of the Intestinal Epithelium.

The intestinal epithelium is made up of diverse cell types organized into microdomains termed the crypt and villus. The microbiome lies adjacent to the intestinal epithelium in close contact with a mucous layer produced by the epithelium. The villus contains differentiated cells including enterocytes, goblet cells, enteroendocrine cells, and tuft cells. The crypt contains the undifferentiated intestinal stem cells (ISCs) and transient amplifying (TA) cells. The Paneth cells and few enteroendocrine and tuft cells reside alongside the ISC in the crypt. The stromal cells reside directly beneath the epithelium in the crypt region and provide essential regulatory factors to the ISC. Created in BioRender.

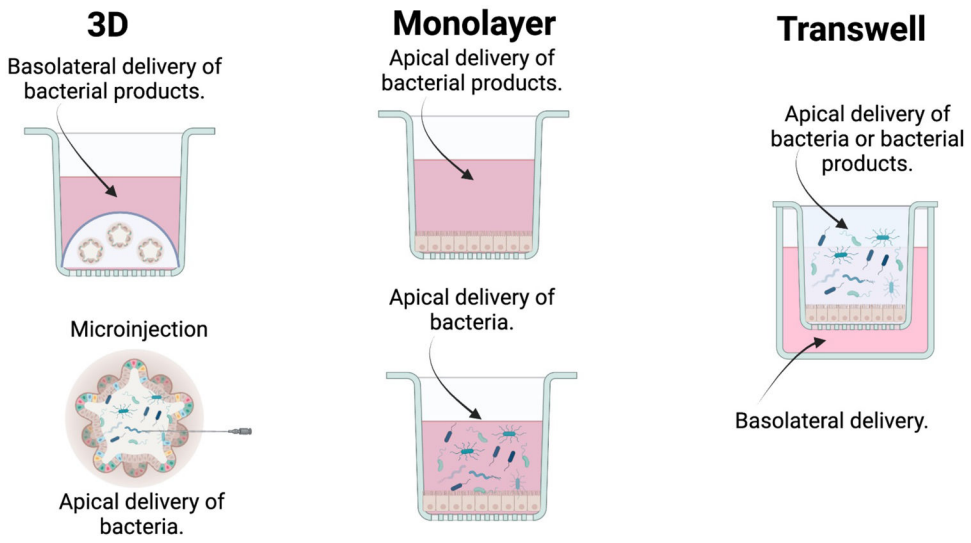

Figure 2: Formats of Human Intestinal Organoids

Human intestinal organoids (HIOs) can be used in a variety of formats depending on the scientific questions to be explored. Created in BioRender.

Table 1.

Summary of the known effects of microbial species and strains on ISC regulation.

Microbe / Strain	Model	Effects on ISCs	Mechanisms	Reference
Akkermansia muciniphila				
ATCC BAA-835 and AK32	Normal mouse, radiation and methotrexate mouse damage model	Stimulates proliferation of Lgr5+ ISCs, promotes differentiation to Paneth cells and goblet cells, promotes gut repair following damage	Activates Wnt/β-catenin pathway by promoting Wnt3 secretion	Kim et al. (2021), Duan et al. (2023)
Bacillus subtilis				
JNFE0126	DSS-induced mouse colitis model	Stimulates proliferation, increases Lgr5+ ISC numbers	Protects ISCs from inflammatory injury; rebalances intestinal microbiota	Zhang et al. (2020)
Bacteroides fragilis				
ZY-312	DSS-induced mouse colitis model	Promotes colonic proliferation	Promotes colonic mucosal regeneration in colitis via IL-22-induced STAT3 phosphorylation	Zhang et al. (2023)
086-5443-2-2	Human colonic epithelial HT29/C1 cells	Promotes proliferation and *c-myc* expression	Activates β-catenin/TCF signaling pathway via	Wu et al. (2003)
Erwinia carotovora carotovora 15				
Ecc15	Ecc15-infected *Drosophila* model	Stimulates ISC proliferation and differentiation to enteroendocrine cells	JAK-STAT pathway induction of ISC proliferation via Upd3 release from enterocytes	Buchon et al. (2009b), Liu et al. (2022)
Lactobacillus acidophilus				
ATCC4356	*Salmonella typhimurium*-infected mouse intestinal organoids	Inhibits proliferation, inhibits excessive differentiation of Paneth cells and goblet cells	Inhibits overactivation of Wnt/β-catenin pathway	Lu et al. (2020)
Lactobacillus casei				
ATCC334	Radiation-induced rat injury model	Stimulates proliferation, increases Lgr5+ ISC numbers	Promotes proliferation via alpha-linolenic acid production and IL-22	Hua et al. (2023)
ATCC334	Normal mouse and porcine epithelial cells	Stimulates epithelial cell proliferation	Induces epithelial expression of *Reg3a* to inhibit pathogens and stimulate damage repair	Bai et al. (2021)
Lactobacillus plantarum				
	Normal *Drosophila*	Stimulates ISC proliferation	Nox1-dependant reactive oxygen species generation	Jones et al. (2013)
Lactobacillus reuteri				
D8	*C. rodentium*-infected mouse, DSS-induced mouse colitis model, TNF-damaged mouse organoids	Stimulates proliferation, induces differentiation of Paneth cells, increases Lgr5+ ISC numbers	Induces R-spondin expression and activates Wnt/β-catenin pathway;	Wu et al. (2020)

Microbe / Strain		Model	Effects on ISCs	Mechanisms	Reference
	D8			Stimulates lamina propria lymphocyte secretion of IL-22 and STAT3 signaling pathway	Hou et al. (2018)
	17938	Normal neonatal mice	Stimulates proliferation	Increases microbial phylogenetic diversity	Preidis et al. (2012)
Lactobacillus rhamnosus					
	GG	Normal mouse colonic organoids, normal mouse	Stimulates colonic epithelial proliferation	Microbe-induced leptin expression triggers JAK-STAT signaling in a *nox1* and leptin receptor-dependent manner	Darby et al. (2020), Jones et al. (2013)
	GG	Normal mouse	Stimulates ISC proliferation	Nox1-dependant reactive oxygen species generation	Lyu et al. (2022)
	GG	Intrauterine growth retardation rat pups	Stimulates proliferation and goblet cell differentiation	Stimulates WNT/β-catenin signaling	Banasaz et al. (2002)

Table 2.

Summary of the known effects of microbial metabolites and factors on ISC regulation.

Microbial factor	Description	Effects on ISCs	Mechanisms	Reference
Butyrate	SCFA	Stimulates proliferation	Stimulates MEK-ERK signaling	Park et al. (2016)
		Stimulates proliferation	Unspecified	Boffa et al. (1992)
		Inhibits proliferation	Unspecified	Kaiko et al. (2016)
Deoxycholic acid	Secondary bile acid	Inhibits proliferation	Possible FXR inactivation of EGFR signaling	Dossa et al. (2016)
Flagellin	Flagella component	Stimulates proliferation	Upregulation of NOX1 and stimulation of EGFR signaling	van der Post et al. (2021)
Indole-3-aldehyde	Tryptophan metabolite	Promotes proliferation and Paneth cell differentiation	Stimulation of IL-22 secretion and STAT3-dependent proliferation stimulation	Hou et al. (2018)
		Promotes proliferation and goblet cell differentiation	Promotes IL-10 signaling	Powell et al. (2020)
Indoleacetic acid	Tryptophan metabolite	Inhibits proliferation	Aryl hydrocarbon receptor (AhR) E3 ubiquitin ligase degradation of β-catenin	Kawajiri et al. (2009)
Lactate	Organic acid	Stimulates proliferation, enhances survival of Lgr5+ ISCs	Gpr81-dependent Wnt3 expression and WNT/β-catenin pathway stimulation	Lee et al. (2018)
Lipopolysaccharide	Gram-negative bacterial cell membrane	Inhibits proliferation, promotes cell death, promotes goblet cell differentiation	TLR4-induced apoptosis	Naito et al. (2017)
Muramyl-dipeptide	Peptidoglycan (cell wall) motif	Stimulates proliferation, enhances survival of Lgr5+ ISCs	Nod2-mediated cytoprotection	Levy et al. (2020), Nigro et al. (2014)
Propionate	SCFA	Stimulates proliferation, increases ISC stemness, increased LGR5+ cells	Stimulates MEK-ERK signaling and WNT signaling	Park et al. (2016), Duan et al. (2023)
Succinic acid	Organic acid	Inhibits proliferation	Unspecified	Inagaki et al. (2007)
Valproic acid	SCFA	Stimulates proliferation, suppresses secretory lineage differentiation	Stimulates Notch pathway	Yin et al. (2014)
		Stimulates proliferation	Stimulates MEK-ERK signaling	Park et al. (2016)