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Host-microbiota interaction in intestinal stem cell homeostasis

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ABSTRACT

Intestinal stem cells (ISCs) play a pivotal role in gut physiology by governing intestinal epithelium renewal through the precise regulation of proliferation and differentiation. The gut microbiota interacts closely with the epithelium through myriad of actions, including immune and metabolic interactions, which translate into tight connections between microbial activity and ISC function. Given the diverse functions of the gut microbiota in affecting the metabolism of macronutrients and micronutrients, dietary nutrients exert pronounced effects on host-microbiota interactions and, consequently, the ISC fate. Therefore, understanding the intricate host-microbiota interaction in regulating ISC homeostasis is imperative for improving gut health. Here, we review recent advances in understanding host-microbiota immune and metabolic interactions that shape ISC function, such as the role of pattern-recognition receptors and microbial metabolites, including lactate and indole metabolites. Additionally, the diverse regulatory effects of the microbiota on dietary nutrients, including proteins, carbohydrates, vitamins, and minerals (e.g. iron and zinc), are thoroughly explored in relation to their impact on ISCs. Thus, we highlight the multifaceted mechanisms governing host–microbiota interactions in ISC homeostasis. Insights gained from this review provide strategies for the development of dietary or microbiota-based interventions to foster gut health.

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1. Introduction

In the intricate ecosystem of the intestines, the dense and diverse community of microbiota plays a pivotal role in regulating various key physiological functions of the host, including intestinal epithelial maturation, modulation of the immune system, and maintenance of metabolic balance.[1] The continuous renewal and differentiation of intestinal epithelial cells driven by ISCs in crypts are essential for maintaining the structural integrity and functionality of the intestines. Therefore, elucidating specific mechanisms regulating ISC homeostasis and investigating the interplay between the microbiota and the host in regulating ISC homeostasis are essential for advancing the understanding of gut health and developing targeted interventions for maintaining intestinal homeostasis.

The crypt–villus structure of the intestine is essential for efficient digestion, absorption, and reliable pathogen resistance.[2] Continual intestinal epithelial cell self-renewal, supported by ISCs inside the crypt, is imperative for coping with persistent luminal challenges.[3] ISCs feed daughter cells into the transit-amplifying compartment, and then TA cells (or progenitor cells) rapidly proliferate and move out of the crypt to differentiate into mature intestinal epithelial cells including absorptive cells (enterocytes and M cells) and secretory cells (Paneth, goblet, enteroendocrine, and tuft cells), each of these cell types carries out unique and specialized functions.[4] The ISC niche regulates the proliferation and differentiation of mammalian ISCs.[5]

Stomach, small intestine, and large intestine harbor distinct microbial communities that participate in tissue homeostasis.[6,7] Intestinal microbiota can regulate ISCs through pattern-recognition receptors (PRRs) or by modulating the redox state and oxygen concentration in the intestine.[8–11] On the other hand, there is growing evidence that bioactive

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metabolites derived from the intestinal microbiota, such as lactate, short-chain fatty acids (SCFAs), and secondary bile acids (SBAs), can influence various physiological functions in the host, including ISC activity.[12,13] Dietary nutrients can be used as an intervention to control the makeup of the intestinal microbiota, serving not only as a source of energy for ISC metabolism and a means of regulating ISC fate through nutrient-sensing pathways,[14–16] but also influence ISC function through interactions between nutrients and the intestinal microbiome.[17] Therefore, gaining a deeper understanding of connections between dietary nutrients, intestinal microbiome, and ISCs, and their regulatory mechanisms, may provide new perspectives on strategies for manipulating the intestinal microbiota to promote intestinal health.

ISC-derived organoids containing all main types of epithelial cells mimic physiological functions of intact intestinal epithelium, including nutrient absorption, ion transport, secretion, and mucus production.[18–20] This advance has addressed limitations of animal studies and cell lines in understanding diet-host and microbiome-host interactions.[21,22] Over the past decade, an increasing number of technologies have been applied to enhance the availability of intestinal organoids, such as 2D culture of organoids,[23] enhanced epithelial polarization,[24] and co-culture of organoids with intestinal immune cells, intestinal mesenchymal cells, and bacteria.[25] In addition, micro-engineered and high-throughput automated organoid culture has enhanced our understanding of the effects and mechanisms of nutrients and microbiomes on ISCs.[26] In this study, we summarize the latest progress in understanding the crosstalk between nutrients, hosts, and intestinal microbiota in regulating ISC homeostasis with the aim of elucidating how the intestinal microbiome regulates host intestinal health.

2. Principal signaling mechanisms in ISC fate regulation

2.1. ISCs

The mammalian intestine harbors two populations of ISCs. One is the crypt base columnar cells (CBCs), also called 'activated ISCs,' which are intercalated with the granular Paneth cells at the crypt base.[27] LGR5 (leucine-rich repeat-containing G-protein-coupled receptor 5) is one of the most prominent target genes of the Wnt signaling pathway and is exclusively expressed in CBCs at the bottom of the crypt.[28,29] Lineage-tracing experiments have demonstrated that LGR5^{+} CBCs meet two criteria for stemness: long-term self-renewal and differentiation into all epithelial lineages.[27] Another population of ISC, known as '+4 ISCs' or 'quiescent ISCs' is situated at the fourth position above the crypt base, expressing markers such as Hopx, Bmi1, mTert, and Lrig1.[30–33] Subsequent studies have shown that + 4 ISCs can undergo rapid proliferation and give rise to active ISCs that promote intestinal epithelial repair when active ISCs are subject to injury conditions.[34,35] Thus, some researchers have defined + 4 ISCs as reserve intestinal stem cells (rISCs) because they can replenish the pool of cycling CBC cells as needed; however, this concept remains controversial. Recent studies have shown that the dedifferentiation of absorptive and secretory progenitor cells is the principle means for ISC restoration.[36,37](Figure 1a)

2.2. Key signaling pathways for ISC fate determination

Unique characteristics and functions of ISCs depend on a supportive microenvironment that includes Paneth cells, intestinal subepithelial myofibroblasts, and intestinal stromal cells.[38] In addition, this microenvironment is regulated by multiple factors, including the endocrine system, intestinal microbes, and enteral dietary nutrients.[38] Several signal pathways, such as Wnt, Notch, and BMP from the ISC microenvironment coordinate to control ISC fate and function[18] (Figures 1b and 2).

2.2.1. Wnt signaling

The Wnt signaling pathway plays an essential role in maintaining ISC proliferation and controlling ISC fate. Wnt ligands are produced by both Paneth cells and the intestinal mesenchymal cells.[39] The binding of Wnt ligands to the Frizzled-LRP5/LRP6 receptor complex prevents the continuous degradation of β-catenin by a multiprotein

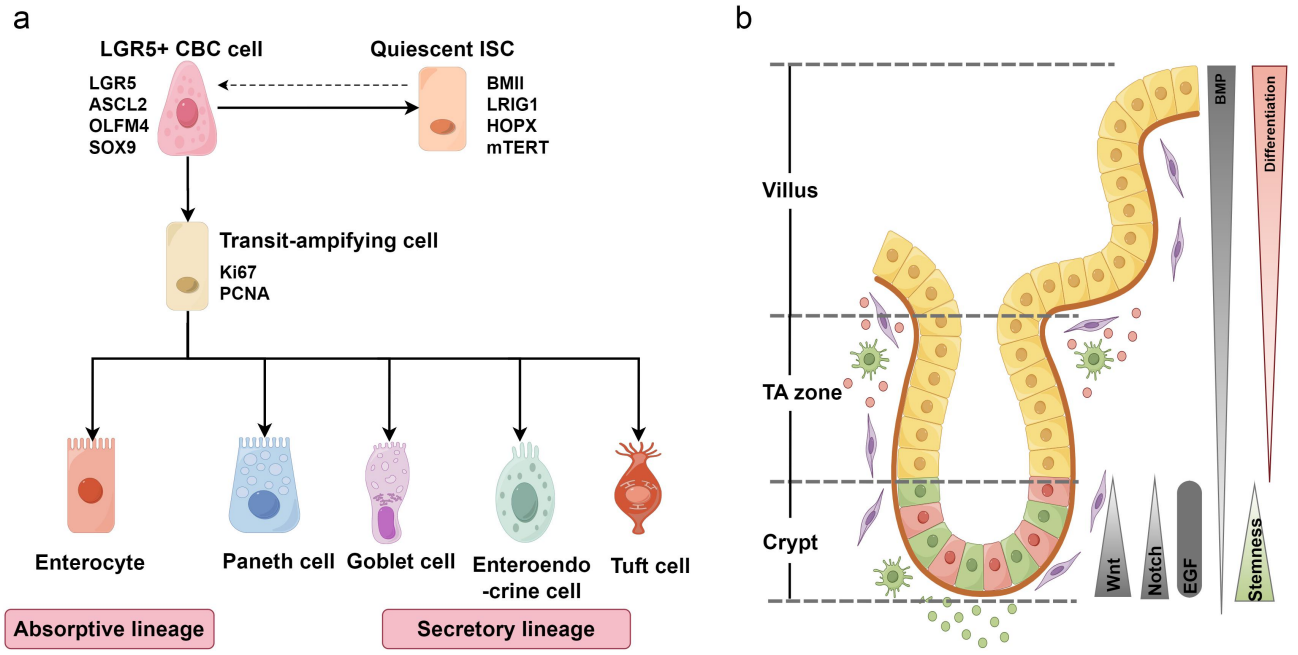

Figure 1. ISCs and differentiated progeny in the small intestine. (a) Active ISCs feed daughter cells into the transit-amplifying compartment, and TA cells differentiate into mature intestinal epithelial cells, including absorptive and secretory cells. Quiescent ISCs can be converted to active ISCs to promote intestinal epithelial repair. (b) Villus-crypt axis structure of the small intestine. Intensity gradient of the four crucial signaling pathways for ISC maintenance along the villus-crypt axis. This figure was drawn using online Figdraw software (https://www.figdraw.com/#/).

'destruction complex' comprising Axin, adenomatous polyposis coli (APC), casein kinase I (CKI), and glycogen synthase kinase 3β (GSK3β).[40] Unphosphorylated β-catenin, which is not degraded, accumulates in the cytoplasm and translocates into the nucleus, where it binds to T-cell factor (TCFs, also called lymphoid enhancer factor, LEF) family of transcription factors to regulate expression of target genes that activate ISCs.[40] Zinc and ring finger 3 (ZNRF3) and Ring finger protein 43 (RNF43), as target genes of Wnt signaling, translocate to the plasma membrane, where they recognize and induce the ubiquitination and degradation of Frizzled through Dishevelled (Dvl), shutting off Wnt signaling. R-spondin, which is an essential cytokine for intestinal organoid culture *in vitro*, binds to LGR4/5 and ZNRF3/RNF43 and further induces ubiquitination and degradation of Wnt receptors to reinitiate Wnt signaling.[41]

Global knockout of Tcf4 in neonatal mice or conditional deletion of Tcf4 in adult mice in the intestinal epithelium contributes to ISC loss, similar to the results observed with overexpression of Dickkopf-related protein 1 (Dkk1), an inhibitor of the Wnt signaling pathway, by adenoviral transfection of the intestinal epithelium or via genetic modification.[42,43] These results suggest that Wnt signaling is essential for the development and maintenance of ISCs.[44,45]

2.2.2. Notch signaling

Notch signaling is important for maintaining the ISC pool and controlling the balance between secretory and absorptive lineages. Direct membrane contact between two cells is essential for the activation of Notch signaling, whereby one cell expresses Notch ligands (such as DLL1 or DLL4) and the other expresses Notch receptors (NOTCH1–4).[46] Once membrane-bound Notch receptors and their ligands bind, Notch signaling is activated. Notch receptors undergo γ-secretase mediated cleavage, ultimately releasing the Notch intracellular domain (NICD) into the cytoplasm.[47] NICD is then transported into the nucleus and binds to CSL (CBF-1/RBP-Jκ, Su(H), Lag-1) to form a transcriptional activator complex resulting in the expression of Notch target genes.

The hairy and enhancer of split (HES) family genes are main target genes of Notch signaling. HES1, HES5, and HES7 proteins are major HES proteins expressed in the intestinal epithelium.[47]

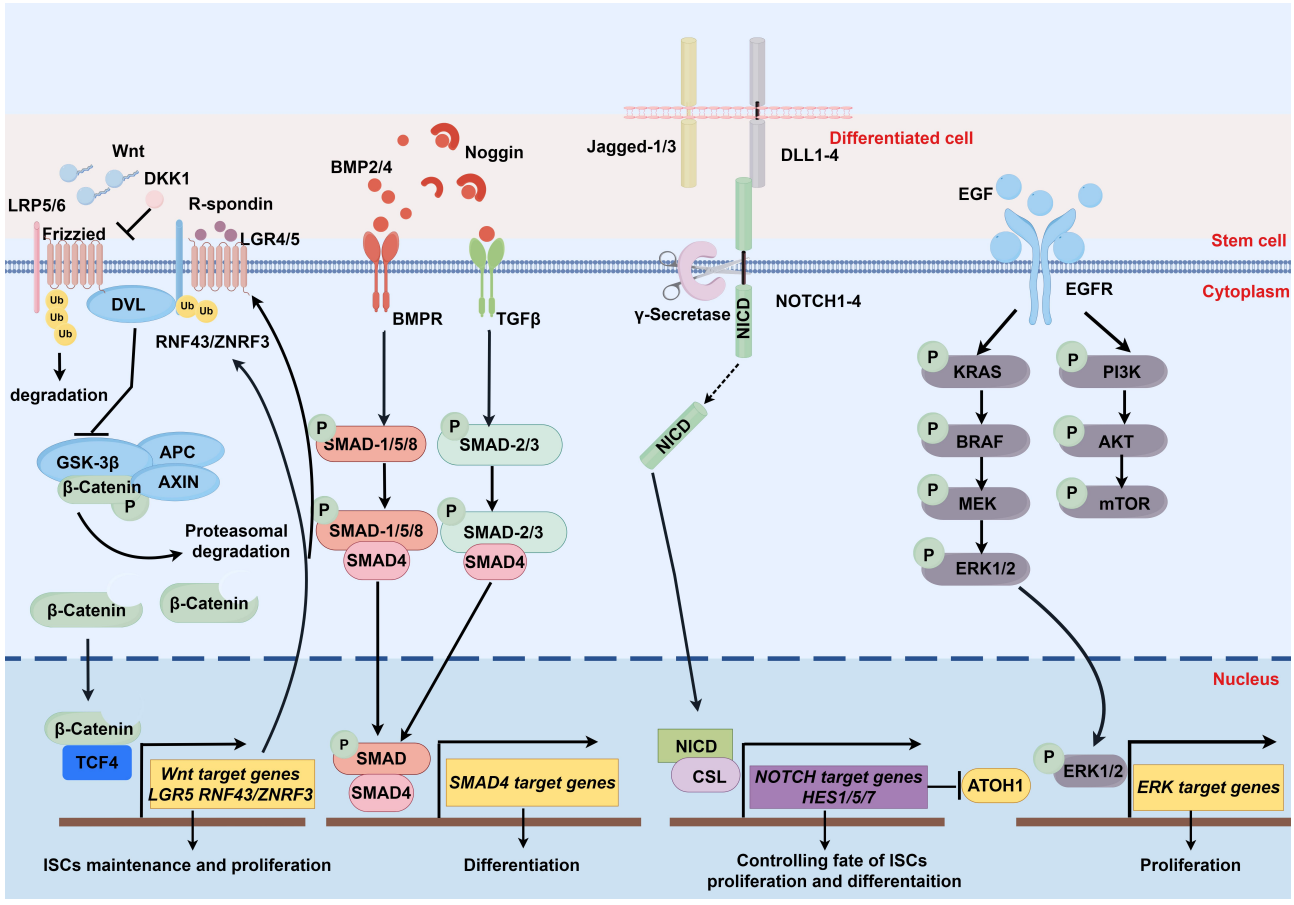

Figure 2. Essential signaling pathway regulating ISC fate. The principal Wnt, Notch, BMP, and EGF signaling cascades collectively regulate ISC behavior and homeostasis. Further details are provided in the main text. This figure was drawn using online Figdraw software (https://www.figdraw.com/#/).

Once expressed HES family proteins repress transcription of another basic helix-loop-helix transcription factor, ATOH1. HES1 null embryos develop secretory cell hyperplasia at the expense of absorptive enterocytes, whereas ATOH1 loss leads to the inability to generate secretory-type cells.[48–50] Hence, ATOH1 plays a role opposite to that of Notch/HES1 in ISC differentiation. Blocking Notch signaling with a Notch antibody induces secretory lineage hyperplasia by repression of the Wnt signaling, while attenuation of Wnt signaling rescues the phenotype associated with Notch blockade.[51] These results indicate that Notch and Wnt signaling jointly regulate ISC activity and differentiation through a negative feedback regulatory mechanism.

2.2.3. EGF signaling

Epidermal growth factor (EGF) is a critical component that drives ISC proliferation. EGF and TGFα (transforming growth factor-alpha) produced by Paneth cells act as ligands for the EGF receptor (EGFR) expressed by CBC stem cells.[39] Overactivation of EGF signaling causes an increase in cell division rate of ISCs and, ultimately, could cause cancer.[52] Therefore, the activity of this pathway must be tightly regulated. Knockout of Lgr1, which serves as a negative feedback regulator of the EGFR in CBC cells, contributes to duodenal adenomas with significant intestinal crypt expansion, emphasizing the importance of EGF signaling in regulating the rate of intestinal epithelial turnover.[31,53] However, EGF signaling appears to be unnecessary for maintaining ISC identity. Blocking EGF signaling leads proliferative ISCs to enter a quiescent state and stops the growth of organoids, whereas restoring EGF signaling enables their reentry into the proliferative state.[54]

2.2.4. BMP signaling

Bone morphogenetic proteins (BMPs) restrict ISC expansion to maintain intestinal homeostasis and prevent ISC hyperproliferation following damage. BMP ligands bind to type II receptors (BMPRII), leading to the phosphorylation and activation of type I receptors (BMPRI).[55] Phosphorylated BMPRI further phosphorylates and activates R-Smads (Smad1, 5, and 8) and forms a complex with a co-Smad (Smad4) to translocate into the nucleus and regulate target gene expression.[55,56] BMP2 and BMP4 are the main ligands for BMP receptors in the small intestine.[57,58] The BMP signal antagonist Noggin, generated by myofibroblasts and smooth muscle cells in the submucosa, is an essential factor for the culture of intestinal organoids *in vitro*, thus emphasizing the importance of BMP signaling in maintaining ISC homeostasis.[18,59]

BMP signals exhibit an increasing concentration gradient along the crypt-villus axis, in contrast to Wnt signaling.[60] Overexpression of the BMP inhibitor Noggin and conditional deletion of BMP's main receptor Bmpr1α lead to crypt expansion.[61,62] These findings resemble the phenotype observed in juvenile patients with polyposis and mutations in the BMP pathway.[63] An earlier study indicated that BMP signaling inhibits the nuclear accumulation of β-catenin to suppress Wnt signaling via the PTEN-PI3K-AKT pathway.[62] Nevertheless, a 2017 study challenged this conclusion by demonstrating that BMP tightly governs ISC expansion via the regulation of stem cell signature genes, including Lgr5, Sox9, and Cdk6, through SMAD-mediated recruitment of HDAC1.[64]

3. Interplay between host and microbiota in ISC homeostasis

The intestinal lumen and mucosa harbor a variety of microorganisms, including bacteria, fungi, archaea, bacteriophages, and protists, that collectively form the gastrointestinal microbiome. In the last two decades, progress in microbial culture and high-throughput sequencing technologies has significantly enhanced our comprehension of the composition and functionality of the intestinal microbiota. Analysis of bacterial communities in the gastrointestinal tracts of different mammals has revealed a coevolutionary relationship between the bacterial community structure and mammalian lineages, resulting in a mutualistic symbiotic ecological structure. In this section, we provide an overview of the latest advancements in understanding the relationship between the intestinal microbiota and the maintenance of ISC homeostasis.

3.1. Immune function

Intestinal epithelial cells and antigen-presenting cells (such as dendritic cells) express numerous types of PRRs, including Toll-like receptors (TLRs) and nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs), which recognize microorganism-associated molecular patterns (MAMPs) in bacteria, including pathogenic bacteria and beneficial symbiotic bacteria.[65,66] Several studies have revealed the crosstalk between the intestinal microbiota and ISCs through PRRs.[8] In addition, the intestinal microbiota regulates ISCs by modulating the redox state and oxygen concentration in the intestine.[9–11]

3.1.1. PRRs

3.1.1.1. Microbiota regulation of ISCs through TLRs. Intestinal epithelial TLR signaling plays an essential role in crypt dynamics by altering ISC proliferation and differentiation. Peptidoglycan (PG) and lipoteichoic acid (LTA), from bacteria including *Lactobacillus* spp., *Bifidobacterium* spp., and *Bacillus subtilis*, facilitate Toll-like receptor 2 (TLR2) signaling.[67] *Lactobacillus rhamnosus* GG (LGG) releases LTA to activate TLR2 on macrophages, thereby protecting ISCs from radiation by stimulating macrophages to secrete chemokines and induce migration of prostaglandin E2 (PGE2)-secreting MSCs.[68] Hou et al. reported that *Bacillus subtilis* induces ISCs differentiation through inhibiting the Notch pathway in an LTA-TLR2-dependent manner.[69] The expression of Toll-like receptor 4 (TLR4) was first observed in $LGR5^{+}$ ISCs, and its activation reduced proliferation and increased apoptosis of ISCs via a p53-up-regulated modulator of apoptosis, both *in vivo* and in ISC-based organoid culture.[70] TLR4 also controls ISC fate by modulating Notch and Wnt signaling. TLR4

has been reported to inhibit Wnt signaling by suppressing the activation of the Wnt receptor LRP6 and blocking the protective effect of Wnt3a ligands.[71] Additionally, deletion of TLR4 in the intestinal epithelium in mice and in intestinal organoids both lead to increased goblet cells.[72] Lipopolysaccharide (LPS) derived from crypt-specific core microbiota (e.g., *Acinetobacter*, *Delftia*, and *Stenotrophomonas*) in mice inhibits ISC proliferation and promotes differentiation of goblet cell lineages in a TLR4-dependent manner, further highlighting the role of microbiota-derived LPS-mediated TLR4 activation of ISCs.[73] In conclusion, the evidence presented above strongly suggests interactions among intestinal microbiota, TLR, Wnt, and Notch signaling pathways, impacting the fate of ISCs .

3.1.1.2. Microbiota regulation of ISCs through NLRs. NOD2, a member of the NLR subfamily, plays a critical role in recognizing conserved bacterial peptidoglycan motifs and triggering host immune responses.[74] MDP (muramyl dipeptide) is a commonly found peptidoglycan motif in all bacteria and activates NOD2.[75] $LGR5^+$ ISCs constitutively express NOD2 at substantially higher levels than Paneth cells within the intestinal crypt,[76] and MDP strongly protects ISCs from oxidative stress-mediated cell death and promotes epithelial regeneration via a NOD2-dependent pathway.[76] A subsequent study further showed NOD2 facilitates a cytoprotective process by the removal of the lethal excess of ROS molecules through mitochondrial mitophagy.[77] This mechanism is activated by synergistic activation of NOD2 and ATG16L1 via a nuclear factor κB (NF-κB)-independent pathway. Another study has demonstrated that NOD2 supports crypt survival and intestinal epithelial regeneration after irradiation-induced ISC damage.[78] These results illustrate intestinal microbe-derived molecules trigger ISC survival to promote intestinal epithelial recovery in a NOD2-dependent manner.

3.1.2. Reactive oxygen species and hypoxia

Multiple studies have suggested that indigenous bacteria in the gut interacts synergistically with reactive oxygen species (ROS) in epithelial cells, while exogenous bacteria produces ROS and upregulates the innate gut immune response, leading to increased ROS generation to shape ISC development.[79] ROS modulate ISC fate by controlling a cascade of signal responses, including Wnt, BMP, and Notch pathways.[80] In addition, ROS autonomously govern various epigenetic changes that impact ISCs. These changes include CpG island methylation, histone acetylation on lysine tails, and deacetylation through SIRTs, all of which contribute to ISC fate.[81,82] It has been conceived that at low ROS concentration, ISC remain inactive and undifferentiated, preserving their stem-like properties. Higher ROS levels promote ISC proliferation and differentiation, while an excessive increase in ROS ultimately triggers apoptosis.[83–86] Recent studies have also demonstrated that some intestinal symbiotic bacteria, such as *Lactobacillus plantarum*, stimulates ROS production to enhance ISC proliferation through activation of the Nrf2/Keap1 pathway both in mice and *Drosophila* .[10,86] However, excessive ROS generation shortens *Drosophila* lifespan.[87]

The intestinal epithelium can experience prolonged hypoxia, with significant pO_2 fluctuations overtime. Such hypoxia can lead to ROS-mediated ISC proliferation. Hypoxia induces ROS production, which stimulates Extracellular regulated kinase 1/2 (ERK1/2) phosphorylation and activates IκB kinase, resulting in the release of NF-κB from IκB and leading to increased hypoxia-inducible factor-1α (HIF-1α) levels.[88,89] Similarly, HIF-1α promotes maintenance of gut barrier and ISC growth.[90–92] It is well established that HIF-1α and ROS interact to maintain ISC homeostasis.[93] Overexpression of HIF-1α functions as a suppressor of ROS production during periods of excessive ISC progeny generation, while HIF-1α knockdown results in higher ROS levels in the Caco-2 intestinal epithelial cells when treated with the hypoxia-inducing agent $CoCl_2$.[94]

3.2. Metabolic interaction

Recent studies have demonstrated that complex metabolic interactions between the intestinal microbiota, their metabolites, and the host are important for maintaining intestinal homeostasis.

Here, we provide a concise overview of key discoveries concerning the impact of intestinal microbiota-derived metabolites on regulating ISC function, aiming to enhance our comprehension of the dynamic equilibrium within the intestines (Figure 3 and Table 1).

3.2.1. SCFAs, lactate, and succinate

SCFAs, comprising approximately 60% acetate, 25% propionate, and 15% butyrate, are the primary end products resulting from the fermentation of complex carbohydrate fibers by anaerobic symbiotic bacteria in the intestines.[114] SCFAs, especially butyrate, serve as an energy source for colonocytes and have various direct or indirect physiological effects on the host, such as the epithelial barrier, immune responses, and energy metabolism. These effects are mediated through their role as ligands for metabolite-sensing G protein-coupled receptors (GPCRs) and histone deacetylases (HDACs) inhibitor.[115–117]

Acetate, the main final metabolite of carbohydrates, has no impact on the growth, proliferation, or passaging capacity of intestinal organoids under physiological conditions. However, acetate supports the formation, growth, and budding of organoids by inhibition of β-oxidation when acetyl-CoA concentration is low.[95,118]

Propionate also regulates ISC function. Propionate supplementation reserves chemical injury-induced loss of aISC markers *LGR5* and *OLFM4* expression.[96] Another research revealed that the supplementation of fucose increases the production of propionic acid by *Akkermansia*

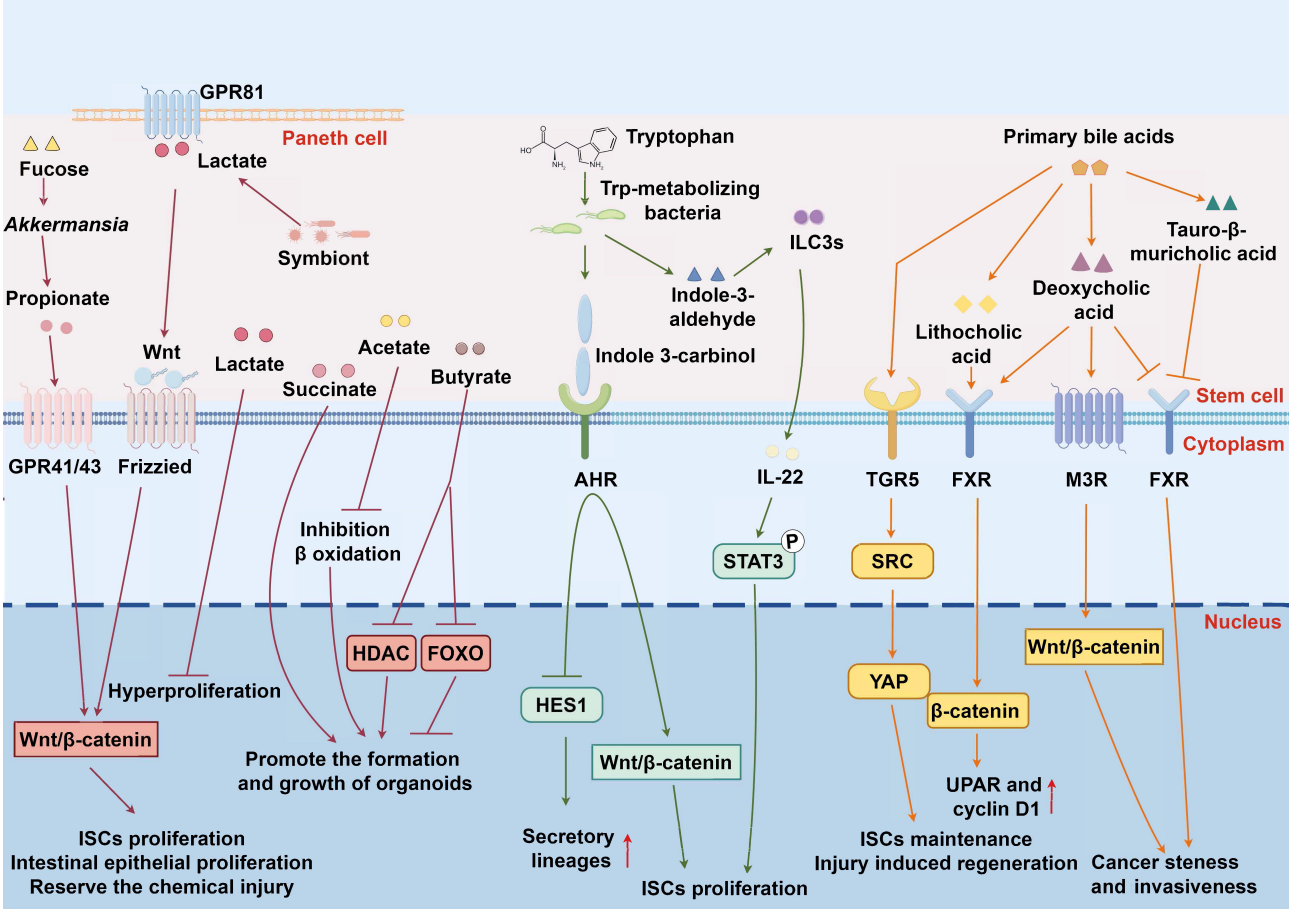

Figure 3. The effects of key microbiota-derived metabolites on ISCs and the pathways that control gut homeostasis. Microbiota-derived metabolites, such as SCFAs, lactate, succinate, indoles and their derivatives, and bile acids, play a crucial role in regulating ISC homeostasis and associated signaling pathways. Further details are provided in the main text. This figure was drawn using online Figdraw software (https://www.figdraw.com/#/).

Table 1. Effects of intestinal microbiota-derived metabolites on ISCs.

Metabolites	Findings	Reference
Acetate	Acetate supports the formation, growth and budding of intestinal organoids by inhibition of β-oxidation when acetyl-CoA concentration is reduced.	95
Propionate	Propionate treatment reserves the chemical injury with loss of expression of ISCs makers *LGR5* and *OLFM4* in the intestinal organoids of mice.	96
	Fucose accelerates intestinal epithelial proliferation in a Gpr41/Gpr43-dependent manner by promoting *Akkermansia*-related propionate metabolism in mice.	97
Butyrate	Butyrate promotes colonic mucosa proliferation in humans.	98
	Butyrate increases $Lgr5^{+}$ ISC number in small intestinal organoids of mice by inhibiting HDAC.	99
	Butyrate suppresses colonic stem cells proliferation at the physiological concentration in the FOXO3-dependent manner.	100
Lactate	Lactate improves epithelial proliferative activity and crypts size of rat cecum.	101
	Microbiota-derived lactate induces enterocyte hyperproliferation in starvation-refed mice.	102
	LAB-type symbiont-derived lactate stimulates ISC proliferation through Wnt/β-catenin signals of in mice.	103
Succinate	Succinate exacerbates the development of ulcerative colitis by inducing mucosal blood flow and generation of superoxide.	104
	Succinate significantly inhibits colonic cell proliferation and reduces crypt size in the colon of rats.	105
	Succinate attenuates intestinal barrier function in mice and pigs.	106,107
Indoleacetic acid	Indoleacetic acid suppresses β-catenin signals through AHR to inhibit ISCs proliferation.	108
Indole 3-carbinol	Indole 3-carbinol promotes ISCs differentiation to secretory lineages by activating Wnt/β-catenin signals and suppressing Notch signals in an AHR-dependent manner in mice.	109
Indole-3-aldehyde	*Lactobacillus* derived-indole 3-aldehyde activates ILC3 cells through AHR ligands to produce IL-22, which further maintains ISCs proliferation in a STAT3-dependent manner in mice.	25
DCA/LCA	DCA and LCA induce cancer stemness in colonic epithelial cells by modulating M3R and Wnt/β-catenin signaling in colon cancer cells.	110
		111
BAs	BAs at the physiological level promote ISCs regeneration and repair after damage via activating TGR5 signaling in mice.	112
T-βMCA and DCA	T-βMCA and DCA induce proliferation and DNA damage in Lgr5+cells through antagonizing intestinal FXR function.	113

muciniphila, which further promotes the stemness of ISCs through a Gpr41/Gpr43-dependent mechanism.[97]

Butyrate, the least abundant of the three main SCFAs, serves as a significant energy source for colonocytes, stimulating ISC proliferation.[115] However, the effects of butyrate on ISC proliferation remain controversial. Yin et al. reported butyrate promotes ISC amplification in intestinal organoids by inhibiting HDAC.[99] However, another study has found that butyrate suppresses colonic stem cell proliferation at physiological concentrations in a FOXO3-dependent manner.[100] One possible reason for these controversial results is that butyrate exerts varying effects on the intestinal epithelial cells, depending on the specific segment of the intestine being studied.

Intermediate metabolites such as lactate and succinate, which are produced as end products by some intestinal microbes under certain conditions, also regulate ISC function.[119] Previous studies have reported that lactate enhances intestinal proliferation in the small intestine and cecum.[101,102] Another study further demonstrated that *Lactobacillus*-derived lactate promotes ISC-mediated intestinal proliferation by activating the Wnt/*β*-catenin pathway in a GPR81-dependent manner.[103] The effect of lactate on ISC-mediated intestinal proliferation may partly explain the positive effects that *Lactobacillus* has on intestinal homeostasis. The exact effect of succinate on intestinal proliferation and homeostasis remains controversial. Some studies indicated that succinate may inhibit intestinal epithelial proliferation and induce mucosal damage in the colon.[104,105] However, Li et al. demonstrated that succinate improves inflammation responses and intestinal barrier function in mice and pigs.[106,107] Further experiments treating animals or intestinal organoids with physiological concentrations of succinate in the intestinal lumen would be more conducive to exploring the effects of succinate on ISC function.

3.2.2. Indoles and derivatives

Tryptophan (Trp), an essential aromatic amino acid, is metabolized either by the gut microbiota through the indole pathway or by the host cells through the kynurenine and serotonin pathways.[120] Indole and its derivatives modulate intestinal epithelial cell physiology, immune homeostasis, ISC function, and neurotransmission by interacting with the aryl hydrocarbon receptor (AhR) on host cells. Intestinal microbiota such as *Escherichia coli*, *Lactobacillus spp.*, and *Serratia marcescens* express tryptophanase, which

metabolizes Trp into indoles and their derivatives, including indole-3-acetaldehyde, indole-3-aldehyde, indole-3-acetic-acid, indole-3-propionic acid and indoleacrylic acid.[121–123] These molecules can activate AhR signaling to directly or indirectly regulate ISC fate.

AhRs are highly expressed in mouse LGR5$^+$ ISCs and play important roles in controlling ISC proliferation.[124] Indole-3-carbinol, as an AhR ligand, promotes ISC differentiation into secretory lineages by activating Wnt/β-catenin signaling and suppressing Notch signaling.[109] In addition, the regulatory effect of indoles and their derivatives on ISCs partly depends on AhR-driven mechanisms in immune cells.[125] Hou et al. also confirmed that *Lactobacillus*-derived indole-3-aldehyde activates innate lymphoid cells type 3 (ILC3) cells through AhR ligands to produce IL-22, which further increases ISC proliferation in a STAT3-dependent manner.[25]

3.2.3. Bile acids

Liver cells synthesize primary bile acids (BAs) from cholesterol, which are then released into the bile ducts and small intestine with glycin or taurine-conjugation to facilitate nutrient digestion and absorption.[126] The majority of BAs are reabsorbed in the terminal ileum and transported back to the liver, while a smaller portion reaches the colon. In the colon, the resident microbiota produces bile salt hydrolase (BSH) to convert BAs into different SBAs, including deoxycholic acid (DCA) and lithocholic acid (LCA).[127] Thus, the composition and structure of colonic microbiota define the BAs signature.[128] BAs regulate host metabolism and intestinal barrier function through several host cell receptors, including farnesoid X receptor (FXR) and Takeda G protein-coupled receptor 5 (TGR5).[126,129]

BAs exhibit a paradoxical effect on ISCs, potentially connected to their ecological niche and dosage. Physiological levels of BAs promote ISC-mediated intestinal epithelium regeneration after injury by activating TGR5 signaling, resulting in the activation of Src and Yes-associated protein (YAP) and their target genes.[112] In addition, BAs reduce intestinal inflammation, which is dependent on the TGR5.[130] These results indicate that BAs have the potential to enhance intestinal barrier function. However, SBAs, especially DCA and LCA, increase colon cancer stemness and invasiveness of colonic epithelial cells by influencing muscarinic 3 receptor (M3R) and Wnt/β-catenin signaling pathways.[110,111] In line with this, some studies have reported that a high fat diet (HFD) increases BA concentrations and further activates the BA-FXR axis to induce hyperproliferation of colon crypts.[113,131] These results indicate that a basal level of BAs maintains ISC proliferation, whereas HFD-induced higher concentrations of BAs may increase the risk of colorectal cancer, further highlighting the importance of a balanced diet.

3.3. Microbiota-nutrient interaction in regulation of ISC homeostasis

Dietary nutrients affect microbial distribution and alter microbial metabolism, thereby regulating the stability of the intestinal microenvironment. Additionally, the intestinal microbiota influences absorption, metabolism, and utilization of nutrients. Nutritional elements ingested by the body are digested, absorbed, and utilized by the host and intestinal microbiota to regulate intestinal health.

3.3.1. Macronutrients

3.3.1.1. Protein. Protein forms and levels affect the structure and metabolism patterns of the intestinal microbiota, which may further modulate intestinal homeostasis. It was discovered that the microbiota in the porcine small intestine exhibited a preference for utilizing peptides over free amino acids for bacterial protein synthesis *in vitro* .[132] Additional research has indicated that the presence of peptide-bound amino acids contributes to the prevalence of *L. amylovorus* and metabolic patterns characterized by lactate production, underscoring the impact of amino acid utilization on intestinal microbial distribution.[133] The intestinal microbiota actively participates in the digestion, absorption, and metabolism of amino acids in the body, as demonstrated by Dai et al., who used subculture and isotope tracing techniques to illustrate that the intestinal microbiota can utilize dietary amino acids to synthesize bacterial proteins.[134,135]

Amino acids are essential nutrients that directly regulate ISC homeostasis. The evidence to date suggests that the gut microbiota affects the metabolic fate of amino acids, including glutamate, glutamine, and arginine,[134, 136, 137] which further regulates ISCs function. Notably, L-glutamate, one of the most abundant amino acids, plays a vital role in balancing amino acids in the body and regulating intestinal function.[138] L-glutamate has been found to regulate ISC fate through complex mechanisms, involving the EGFR-ERK-mTORC1 pathway[139] and the amplification of β-catenin through the switching of the membrane receptor Frizzled7.[140] In *Drosophila*, L-glutamate stimulates ISC fate through regulating calcineurin and CREB-regulated transcriptional co-activator via Ca^{2+} signaling.[141] Glutamine, another crucial amino acid, serves as the preferred energy substrate for intestinal epithelial cells, promoting ISC activity to accelerate intestinal epithelial regeneration through enhanced Wnt signaling.[142] Similarly, L-arginine and L-methionine have been shown to influence ISC function in response to injury, regulating the ISC niche, proliferation, and differentiation balance.[143–147] In *Drosophila*, methionine and its derivative S-adenosylmethionine reduce midgut mitosis by controlling protein synthesis autonomously in ISCs and induction of the JAK/STAT ligand Unpaired 3 non-autonomously in enterocytes (ECs).[148] These findings underscore critical roles of amino acids in sustaining intestinal epithelial homeostasis.

3.3.1.2. Carbohydrate. Food rich in dietary fiber alters composition of the intestinal microbiota.[149] It can promote colonization of fiber-degrading microbiota and the production of SCFAs, which in turn support intestinal mucosal homeostasis and host health.[149] Conversely, dietary fiber deficiency decreases the abundance of fiber-degrading microbiota in the intestine, potentially leading to the generation of mucus-degrading enzymes by the intestinal microbiota, thereby impairing the mucus barrier.[150] In addition, hindgut nutrient substrate availability, especially the ratio of carbohydrate/nitrogenous compounds, alters microbe-related SBAs metabolism and modulates intestinal barrier function.[151] These studies suggest that carbohydrates, as essential nutritional substrates, modulate the composition and metabolic patterns of the gut microbiota.

Additionally, human milk oligosaccharides (HMO) play an essential role in intestinal development and maturation of neonates.[152] Clinical studies demonstrate that fucosylated HMO such as α2'-fucosyllactose supplementation modifies intestinal microbiota profile in infants and adults.[153,154] Furthermore, feeding sialylated oligosaccharides to newborn piglets increases intestinal crypt depth and proliferation, and reduces diarrhea rate.[155] Interestingly, when specific microbial-colonized mice were fed a Malawian diet along with sialylated oligosaccharide supplementation, early growth and development were improved.[156] Notably, this effect cannot be observed in germ-free mice, highlighting the involvement of the intestinal microbiota in the regulation of intestinal growth and development by sialylated oligosaccharides.

3.3.1.3. Lipid. The interaction between intestinal microbiota and saturated fatty acids plays a vital role in host health under various physiological conditions. HFD increases the relative abundances of *Clostridium*, *Turicibacter*, and *Peptostreptococcaceae*, while notably decreasing the relative abundances of *Bifidobacterium*, *Allobaculum*, and *Bacteroides*.[157] Supplementation with long-chain saturated fatty acids led to increased relative abundances of total *Lactobacillus* species and *Lactobacillus rhamnosus*, which reduced alcohol-induced liver injury in mice.[158] Nevertheless, administering a high saturated fatty acid diet to normal mice increases the abundance of hydrogen sulfide-producing *Desulfovibrio* in fecal samples and colonic permeability, ultimately resulting in mesenteric inflammation.[159]

The intestinal microbiota affects the availability of lipids in the gut. Germ-free mice fed an HFD have higher levels of lipids in the feces than normal mice.[160] Additionally, antibiotic-treated rats exhibited a reduction in lipid content in the lymphoid tissue after being subjected to HFD.[161] In *Drosophila*, HFD induces transient activation of ISCs through modulating the composition of indigenous microbiota.[162] The availability of environmental lipids and the fundamental processes of

fatty acid metabolism significantly influence ISC function. Various dietary components, such as arachidonic acid, beta-hydroxybutyrate, have been shown to influence various signaling pathways and metabolic programs in ISCs, affecting their self-renewal, differentiation, and susceptibility to tumor formation.[163–166] For example, cholesterol has been shown to control ISC differentiation toward the endocrine lineage by modulating Notch signaling in an Hr96-dependent manner.[167] In addition, cholesterol has been shown to be necessary for ISC mitosis by acting as a precursor of steroid hormones and its abnormal intracellular trafficking leads to gut dysbiosis in *Drosophila*.[168] Notably, excess delivery of external lipids, fatty acids, or cholesterol through the diet renders mice ISCs more susceptible toward intestinal tumor formation.[169]

3.3.2. Micronutrients

3.3.2.1. Vitamins. Vitamins are essential cofactors for myriad of enzymes involved in fat and carbohydrate metabolism. Studies have indicated that certain vitamins, when administered in high doses or targeted to the large intestine, can positively impact on the gut microbiome. This includes increasing the abundance of presumed commensals, enhancing microbial diversity and richness, and promoting SCFA production.[170]

Humans lack the biosynthetic capacity for most vitamins, and these must thus be provided exogenously through diet and synthesis by the intestinal microbiome.[171] Previous studies have shown that intestinal microbiota can synthesize vitamins C and K and the B group vitamins.[172,173] In monogastric animals, vitamins produced by the intestinal microbiota are primarily absorbed in the colon, whereas dietary vitamins are absorbed in the proximal small intestine.[174,175] Vitamin A and its metabolite, retinoic acid, enhance ISC stemness and promote ISC differentiation, respectively,[176,177] whereas vitamin B9 rescues the reduction in cell metabolic activity in small intestinal organoids caused by the chemotherapeutic agent methotrexate.[178] The effects of 1,25-dihydroxyvitamin D3 (vitamin D3) on ISC function are controversial, with studies reporting contrasting impacts.[179,180] Vitamin D receptor in enterocytes in the intestine of *Drosophila* is essential for ISC proliferation and enteroendocrine cell differentiation.[181] Vitamin B7 is also critical for ISC maintenance and tumorigenesis in *Drosophila*. In particular, biotin provided through the diet or the microbiota is necessary for mitosis and homeostasis maintenance in ISCs and its absence leads to gut dysbiosis in *Drosophila* .[182] These results underscore the vital role of dietary vitamins and microbial metabolism in regulating ISC homeostasis.

3.3.2.2. Minerals. Dietary mineral deficiencies influence the composition and behavior of the intestinal microbiota. For instance, magnesium deficiency alters the composition of the intestinal microbiota and induces anxiety-like behavior,[183] while zinc deficiency causes taxonomic alterations and decreases overall species richness and diversity in the cecum of broiler chickens.[184] Supplementation of zinc-amino acid conjugates in mice with zinc deficiency during pregnancy rescued the abnormal microbiota composition and gut physiology status.[185] *Parabacteroides* and *Lactobacillus* show a negative correlation with increased iron stores, while members of the Clostridia class exhibit a positive correlation with iron stores.[186] These results indicate that dietary mineral availability directly influences the intestinal microbial composition and metabolism.

Minerals in food can exist in free or bound forms, with free minerals being directly absorbed and bound minerals being released slowly and absorbed by digestive enzymes and the intestinal microbiota. The probiotic *Lactobacillus plantarum 299 v* increases non-heme dietary iron absorption.[187] Dietary supplementation with iron and Vitamin A increases villus height and intestinal surface area in suckling piglets.[188] Selenium-enriched *Bifidobacterium longum* can biotransform inorganic selenium (Na_2SeO_3) into more bioactive organic selenium forms (e.g., selenomethionine [Se-Met]) for efficient utilization by the host.[189] Se-Met notably elevates the population of $LGR5^+$ and $PCNA^+$ cells and concomitantly increases the number of goblet cells, Paneth cells, and absorptive cells compared with deoxynivalenol (DON) treatment alone.[190] Additionally, zinc L-aspartate, in particular Zn, enhances ISC activity to safeguard the integrity of the intestinal epithelium against DON by

activating the Wnt/β-catenin signaling pathway in *in vivo* (mouse) and *ex vivo* (mouse enteroid) models.[191] These findings indicate that the intestinal microbiota is essential for the absorption and metabolism of minerals in the intestine, as well as for maintaining ISC homeostasis.

4. Intestinal organoids: a model for investigating the effect of host – microbiota interaction on ISC homeostasis

In 2009, Clevers et al. first established a murine intestinal organoid model using single LGR5^{+} ISCs from mouse intestinal crypts *in vitro* .[18] Subsequently, the human intestinal organoid model was successfully established by the same group.[192] After 15 years, the technology for intestinal organoid cultures derived from ISCs has become increasingly advanced and has been applied to various experimental animals including rats,[193] rabbits,[194] pigs,[195] chickens,[196] cows,[197] and sheep[198] (Figure 4a).

4.1. Advantages of intestinal organoids

Intestinal organoids contain major types of epithelial cells, which can mimic physiological functions such as nutrient absorption, transport and secretion.[18–20] Additionally, intestinal organoids can maintain more stable phenotypic and genetic characteristics during continuous passage than intestinal cell lines.[199,200] Primary cells and ex *vivo* xenografts have a low expansion potential and are not amenable to cryopreservation and thawing, preventing their widespread use in mechanistic research.[201] Compared to animal models, intestinal organoids are easier to manipulate with shorter culture cycles, and reduced ethical concerns.[19] These advantages make them an excellent model for nutritional and microbial

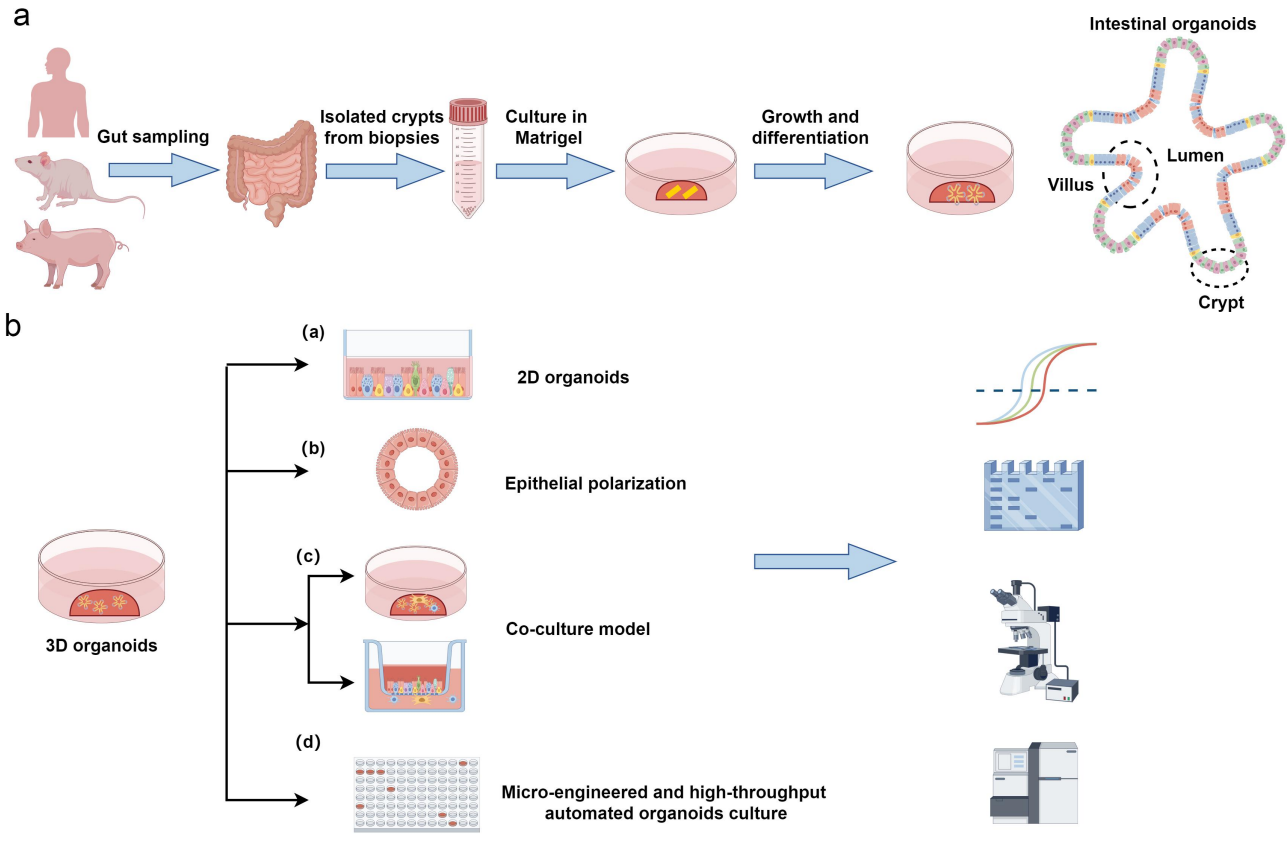

Figure 4. The establishment and engineering improvement of the intestinal organoid model. (a) Flowchart of the establishment of the mammalian intestinal organoid model. Intestinal crypts were isolated from intestinal tissue, and further embedded in Matrigel® with culture medium to form intestinal organoids. (b) Engineering improvement of the intestinal organoid model. (a) 2D organoids; (b) intestinal organoid polarization; (c) co-culture of intestinal organoids with intestinal mesenchymal and immune cells. (d) High-throughput automated organoid culture. Phenotypic analysis, RT-PCR, imaging, single-cell RNA sequencing, and other indicators can be used to evaluate organoid function. This figure was drawn using online Figdraw software (https://www.figdraw.com/#/).

research. Intestinal organoids have been widely utilized to study the impact of diet patterns and nutrients on intestinal health, nutrient transport and absorption functions, interactions between the microbiota and host, and location-specific functions of the intestine.

4.2. Limitations of intestinal organoids

Although intestinal organoids simulate the physiological structure of the intestine, some limitations and challenges remain. The 3D geometric architecture and apical membrane face the inside of the organoid structure to prevent direct contact between nutrients, intestinal microbiota, and bioactive and toxic compounds in the apical epithelium.[202,203] As organoids grow, the efficiency of nutrient supply and waste removal decreases, and the organoids must be re-fragmented and reseeded. In addition, limited one-week lifespan of organoids is inadequate for robust differentiation into the full spectrum of differentiated cell types found *in vivo* .[204] In addition, conventional organoid models lack mesenchymal cells and immune cells derived from various non-epithelial lineages.[205] However, epithelial development, homeostasis, and disease rely on intricate interactions between different cell types to establish and sustain normal intestinal physiological functions, making intestinal organoid model insufficient in mimicking all aspects of intestinal biology. In recent years, various strategies have been developed to overcome these limitations (Figure 4b).

4.3. Engineering improvement of intestinal organoids

4.3.1. Human intestinal/colonic organoids (HIOs/HCOs)

The methods for generating Human pluripotent stem cell (hPSC)-derived small intestinal organoids (HIOs) were first established in 2011,[206] enabling research on human development,[207] modeling genetic intestinal diseases,[208] understanding enteric pathogenesis,[209] and elucidating mechanisms of intestinal physiology.[210] Helmarth et al. further developed an in *vivo* HIO engraftment model to generate mature and functional human intestinal tissues,[211] while also containing a functional enteric nervous system (ENS)[212]and immune cells.[213] A notable limitation in the widespread adoption of hPSC-derived gastrointestinal organoid technologies is the requirement for initial differentiation of hPSCs and reliance on spontaneous morphogenesis to form detached spheroids.[206] To address this challenge, Mayhew et al. introduced a straightforward, reproducible, and scalable approach for generating HIOs through aggregating cryopreservable hPSC-derived mid-hindgut endoderm (MHE) monolayers, significantly enhancing HIO production by approximately tenfold.[214] Given the high incidence of diseases that impact the large intestine such as colitis and colon cancer, Wells et al. detailed the differentiation of human colonic organoids (HCOs) from hPSCs through transient activation of BMP signaling. This innovative approach further expands the utility of HCO technology in studying colonic pathologies.[215]

4.3.2. 2D culture of organoids

Microinjection and mechanical disruption of intestinal organoids into fragments enable direct contact between the apical surface and luminal nutrients and microbes.[77,216,217] However, ensuring synchronous exposure and uniform injection volumes poses a significant challenge.[218] Therefore, some researchers have established 2D monolayer from intestinal organoids by mechanically disrupting or partially enzymatically dissociating 3D organoids and subsequently seeding organoid fragments into tissue culture plates or Transwells, enabling the study of intestinal epithelial permeability and responses to nutrients and microbiome.[219–221] 2D monolayers have been employed in a range of studies of intestinal barrier function, nutrient absorption, and pathogenic infections.[23,139,222] However, previously reported methods have resulted in slower growth and higher variability between different wells.[202] Moreover, production and maturation of 2D monolayers require more single cells and several days of culture.[24] To rapidly obtain a confluent and stable monolayer of cells, several parameters, such as seeding density and culture time, need to be standardized.

4.3.3. Epithelial polarization

Another technique to allow direct contact between the apical side of the intestinal organoids and the

experimental treatment is to reverse the polarity of the enteroids. Co et al. successfully generated apical-out enteroids for the first time by removing the extracellular matrix proteins and suspension culture.[24] Apical-out enteroids still maintain epithelial barrier integrity and functional characteristics of enteroids.[24] Additionally, the apical side of the epithelium is readily accessible for interactions with nutrients and pathogens.[24] Subsequently, Li et al. applied this method to preserve the epithelial polarity of porcine jejunal enteroids and investigated the interactions between a transmissible gastroenteritis virus and the intestine.[223] However, approximately 20% of the organoids failed to preserve epithelial polarity, suggesting phenotypic variability under specific culture conditions.[223]

4.3.4. Co-culture of organoids with intestinal mesenchymal and immune cells

Recent studies demonstrated that intestinal stem cell niches including intestinal mesenchymal cells,[224,225] Paneth cells,[39] and immune cells[226,227] play a crucial role in regulating ISC fate. Hou et al. discovered that L-arginine treatment did not directly target ISCs but rather increased ISC function by stimulating the secretion of Wnt2b by $CD90^+$ stromal cells.[144] $Lepr^+$ mesenchymal cells surrounding intestinal crypts sense dietary changes and maintain ISC function via the leptin-Igf1 axis.[225] *L. reuteri* D8 accelerated ISC regeneration to maintain the intestinal barrier by inducing lamina propria lymphocyte secretion of IL-22.[25] Utilizing an *in vitro* co-culture model of organoids with immune or mesenchymal cells offers an in-depth and systematic approach to understanding the mechanisms by which luminal active substances, such as nutrients and microbiota, affect ISC activity.

4.3.5. Micro-engineered and high-throughput automated organoid culture

Micro-engineered and high-throughput automated organoid culture technologies have been used to address complex biological problems. An image-based screening platform for organoids cultured from single cells has been developed to characterize the phenotypic landscape of organoid development.[26] Organoid microarrays dynamically simulate the functional units of human tissues and organs *in vitro* by combining microfluidic microarray technology with 3D organoid culture technology.[228] Different cells or microorganisms can be added to study cell-cell and cell-microbe interactions.[228] Researchers have also developed a microfluidic platform called IFlowPlate that can be used to culture 128 colon organoids *in vitro*, providing new possibilities for modeling relevant diseases and screening potential therapeutic targets.[229]

5. Conclusions and perspectives

Intestinal homeostasis is maintained by a dynamic interplay between ISC self-renewal and differentiation, which is directly or indirectly regulated by the ISC niche, enteral microbiota, and nutrients. In this review, we provide a detailed discussion of various mechanisms through which host-microbiota interactions regulate ISC function, including immune function, metabolic interactions, and the interplay between microbiota and dietary nutrients, including macronutrients and micronutrients. Furthermore, we provide a summary of the most recent advances in intestinal organoid modeling techniques and their potential applications in the study of nutrients, microbiota, and intestinal health.

Further research should explore the specific mechanisms by which nutrients modulate ISC fate and how gut microbes mediate these effects. It is worth noting that the gut microbiota may indirectly influence the stem cell niche by modulating other cells, such as the immune and nervous systems.[230] Thus, further investigations are needed to explore and improve organoid models, including more accurately mimicking the intestinal microenvironment and enhancing model complexity and diversity to simulate the complexity of interactions between the gut microbiota and the host.

List of abbreviations

ACC1	Acetyl-CoA-carboxylase
AHR	Aryl hydrocarbon receptor
APC	Adenomatous polyposis coli
BAs	Bile acids
BMPs	Bone morphogenetic proteins
BSH	Bile salt hydrolase
CBCs	Crypt-base columnar cells
CSL	CBF-1/RBP-Jκ, Su(H), Lag-1
DCA	Deoxycholic acid

Dkk1	Dickkopf-related protein 1
DON	Deoxynivalenol
ECs	Enterocytes
EEs	Enteroendocrine cells
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
ERK1/2	Extracellular regulated kinase 1/2
FXR	Farnesoid X receptor
GCPRs	G protein-coupled receptors
HADCs	Histone deacetylases
HCOs	Human colonic organoids
HES	Hairy and Enhancer of split
HFD	High-fat diet
HIF-1α	Hypoxia-inducible factor-1α
HIOs	HPSC-derived small intestinal organoids
HMO	Human milk oligosaccharides
HPSC	Human pluripotent stem cell
ILC3	innate lymphoid cells type 3
ISCs	Intestinal stem cells
LCA	Lithocholic acid
LGR5	Leucine-rich repeat-containing G-protein-coupled receptor 5
LPS	lipopolysaccharide
LTA	Lipoteichoic acid
M3R	muscarinic 3 receptor
MAMPs	Microorganism-associated molecular patterns
MDP	Muramyl dipeptide
MHE	Mid-hindgut endoderm
NF-κB	Nuclear factor κB
NICD	Notch intracellular domain
NLRs	Nucleotide-binding oligomerization domain (NOD)-like receptors
PG	peptidoglycan
PGE2	Prostaglandin E2
PPARα	Peroxisome proliferator-activated receptor alpha
PPARδ	Peroxisome proliferator-activated receptor delta
PRRs	Pattern recognition receptors
rISCs	Reserve intestinal stem cells
RNF43	Ring finger protein 43
ROS	Reactive oxygen species
SBAs	Secondary bile acids
Se-Met	Selenomethionine
SFCAs	Short-chain fatty acids
TCFs	T-cell factors
TGFα	Transforming growth factor-a
TGR5	Takeda G protein-coupled receptor 5
TLR2	Toll-like receptor 2
TLR4	Toll-like receptor 4
TLRs	Toll-like receptors
Trp	Tryptophan
YAP	Yes-associated protein
ZNRF3	ZNRF3

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Author contributions

WZ conceived and designed this review, edited the manuscript, and secured funding for the study. HW wrote the manuscript draft. CM participated in the concept development, edited and revised the manuscript. LX helped collect literature and draw the diagram. KY provided advice on the review structure. LS edited and proofread the manuscript. All authors contributed to the article and approved the final version of this manuscript.

Data Availability statement

Data sharing is not applicable to this article, as no datasets were generated or analyzed in the current study. Figures 1–4 were created using Figdraw software (www.figdraw.com).

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