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Modulation of intestinal stem cell homeostasis by nutrients: a novel therapeutic option for intestinal diseases

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Abstract
Intestinal stem cells, which are capable of both self-renewal and differentiation to mature cell types, are responsible for maintaining intestinal epithelial homeostasis. Recent evidence indicates that these processes are mediated, in part, through nutritional status in response to diet. Diverse dietary patterns including caloric restriction, fasting, high-fat diets, ketogenic diets and high-carbohydrate diets as well as other nutrients control intestinal stem cell self-renewal and differentiation through nutrient-sensing pathways such as mammalian target of rapamycin and AMP-activated kinase. Herein, we summarise the current understanding of how intestinal stem cells contribute to intestinal epithelial homeostasis and diseases. We also discuss the effects of diet and nutrient-sensing pathways on intestinal stem cell self-renewal and differentiation, as well as their potential application in the prevention and treatment of intestinal diseases.

Key words: **Intestinal stem cells: Self-renewal: Differentiation: Dietary patterns: Intestinal diseases**

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Introduction

Diet is one of vital lifestyle factors that has a profound impact on intestinal health and disease[1]. Growing evidence suggests that excessive nutrients such as fat and protein lead to intestinal inflammation and therefore may potentiate tumorigenesis[2,3]. In contrast, energy restriction or fasting has beneficial effects on health, including extending life span, slowing aging process and reducing colorectal cancer (CRC) incidence[4–6]. Recent studies have proven that diets and nutritional status directly affect intestinal homeostasis at least in part by regulating intestinal stem cell (ISC) self-renewal and differentiation[3,7–13]. For example, Cheng *et al*. reported that a ketogenic diet enhances ISC self-renewal and promotes post-injury regeneration, but a glucose-supplemented diet has opposite effects[8]. Therefore, a better understanding of the relationship between diet and ISCs as well as their regulatory mechanisms may provide new strategies for preventing and treating intestinal diseases.

ISCs are a group of cell populations located at the base of intestinal crypts that are responsible for the rapid renewal of intestinal epithelium[14]. Similar to other stem cells, ISCs have the ability of self-renewal (a process that expands the stem-cell pool, also called proliferation) and differentiation into enterocytes or secretory-lineage cells[14,15]. These processes can form intestinal organoids (also called min-guts) with 3D structure *in vitro*. The balance between self-renewal and differentiation of ISCs is essential for intestinal epithelial homeostasis, which can be regulated by dietary patterns and nutrients[8,16–19]. Diverse dietary patterns, including caloric restriction (CR), fasting, high-fat diets (HFDs), ketogenic diets and high-carbohydrate diets, directly affect ISC biology or niches via nutrient-sensing pathways, such as mammalian target of rapamycin (mTOR) or AMP-activated kinase (AMPK)[3,8,10,11]. In addition, dietary nutrients, including amino acids, fatty acids, vitamins and gut microbial metabolites, have been implicated in the maintenance of ISC homeostasis[16,18–20]. In this review, we first give an overview of the role of ISCs in intestinal homeostasis and diseases. Then, we summarise recent research about dietary patterns and nutrients influencing ISC self-renewal and differentiation as well as their potential application in therapeutic interventions for intestinal diseases.

Abbreviations: AMPK, AMP-activated kinase; *APC*, adenomatous polyposis coli; cADPR, cyclic ADP-ribose; CR, caloric restriction; CRC, colorectal cancer; CSC, cancer stem cell; DSS, dextran sodium sulphate; HFD, high-fat diet; Hmgcs2, mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2; IGF-1, insulin-like growth factor 1; ISC, intestinal stem cell; mTOR, mammalian target of rapamycin; NAD, nicotinamide adenine dinucleotide; Nampt, nicotinamide phosphoribosyl transferase; PPAR, peroxisome proliferator-activated receptor; TA, transit amplifying.

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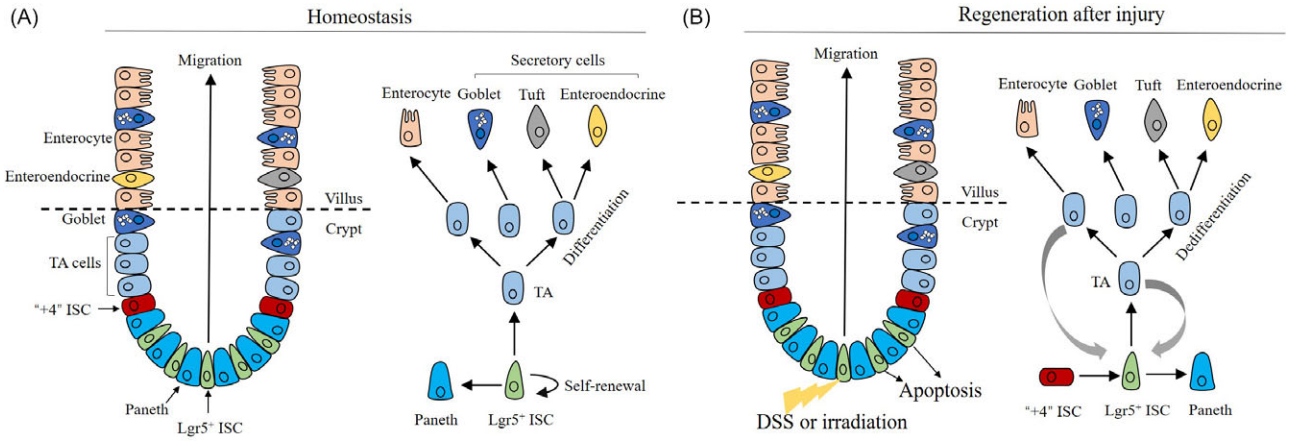

Fig. 1. A model of intestinal stem cell-driven epithelial renewal during homeostasis and regeneration. (A) In the small intestine, Lgr5$^+$ ISCs are located at the crypt base and divide to generate daughter cells and proliferating transit-amplifying (TA) cells, which then differentiate into the various mature cell types, including enterocytes, enteroendocrine cells, goblet cells, tuft cells and Paneth cells in the crypt and villi. (B) Acute injury that leads to the loss of the Lgr5$^+$ ISCs triggers a regenerative response to restore intestinal epithelial renewal. Some stimulations, such as irradiation and DSS, induce Lgr5$^+$ ISC apoptosis, but retain the damage-resistant '+4' ISCs and the Paneth cell precursors. The '+4' ISCs are activated upon injury and rapidly produce Lgr5$^+$ ISCs or committed progenitor cells. Surviving non-stem cells from various lineages also retain stem cell potential and can dedifferentiate into Lgr5$^+$ ISCs to restore intestinal epithelial renewal.

ISCs: roles in intestinal homeostasis and diseases

ISCs and intestinal homeostasis

The intestinal epithelium is a rapidly renewing tissue in which the intestinal epithelial cells turnover every 3–5 d. This rapid renewal is maintained by ISCs residing in the crypts of Lieberkühn that generate daughter cells to differentiate and migrate up towards the crypt–villus axis (Fig. 1A)[21]. Two populations of ISCs have been identified in the mammalian intestine. One is the crypt base columnar cells, also called 'activated ISCs', which are intercalated with Paneth cells at the bottom of the crypt. The key marker for this population is Lgr5, a target gene of the Wnt signaling pathway[21]. The discovery of Lgr5 as a specific marker for this compartment has promoted tremendous advances in the molecular characterisation of ISCs[21].The second ISC population is located at the fourth position above the base of the crypt and is also called '+4' ISCs[22] (Fig. 1A). These '+4' ISCs are a quiescent population of stem cells and are resistant to radiation-induced intestinal injury[23]. Hopx, Bmi1, mTert and Lrig1 have been identified as markers of these '+4' ISCs[24–26].

In the homeostatic small intestine, Lgr5$^+$ ISCs continue to divide to generate their daughter ISCs and proliferative progeny cells known as transit amplifying (TA) cells (Fig. 1A)[27]. TA cells can continuously migrate toward the tip of the villi and differentiate into mature cell types that fulfil the various intestinal functions (Fig. 1A)[14,15]. Enterocytes occupy most of the villi and are highly polarised with a dense apical brush border critical for nutrient absorption. Other cell types contain mucin-producing goblet cells, hormone-secreting enteroendocrine cells and tuft cells, which are involved in regulating immune response against pathogens[28]. Paneth cells located at the crypt base not only provide niche factors for ISCs, but also secrete antibacterial agents to prevent pathogen infection[29]. Lgr5$^+$ ISCs are responsible for intestinal epithelial renewal under normal physiological conditions, and the '+4' ISCs are involved in intestinal regeneration following injury (Fig. 1B)[14]. Acute injury leads to a reduction in the proliferating Lgr5$^+$ ISC pool, but retains the damage-resistant '+4' ISCs and the Paneth cell precursors[30]. The '+4' ISCs are activated upon injury and rapidly produce Lgr5$^+$ ISCs to restore the renewal of the intestinal epithelium (Fig. 1B)[30]. In addition, evidence has reported on the conversion of various cell types following damage to the stem cells, and this process is called dedifferentiation. These cell types, including enterocyte precursors, secretory progenitors and other TA cells, also retain stem cell potential and are consequently converted into Lgr5$^+$ ISCs to restore intestinal epithelial renewal (Fig. 1B)[14]. These data suggest that mature cell types within the intestinal epithelium can dedifferentiate and act as an alternative source of stem cells upon tissue damage.

ISCs and intestinal diseases

Intestinal diseases are closely interconnected with the imbalance between self-renewal and differentiation of ISCs[31]. Lgr5$^+$ ISCs not only act as stem cells for epithelial renewal, but also are identified as the cells of origin for a large proportion of CRCs[32]. CRCs are the common intestinal diseases in humans and are mainly initiated by mutation of the *adenomatous polyposis coli* (*APC*) gene, resulting in nuclear accumulation of β-catenin[33]. Deletion of the *APC* gene in Lgr5$^+$ ISCs leads to early tumorous lesions and metastasis, while *APC* deletion in TA cells does not form adenomas[32], suggesting that Lgr5$^+$ ISCs accumulate some of the early mutations and may serve as early target cells for CRCs. In addition, a population of cells with similar properties of ISCs, such as multilineage differentiation and self-renewal, are found in CRCs and are called colon cancer stem cells (CSCs)[34]. It is worth noting that the gene expression profiles of CSCs have high similarity with that of Lgr5$^+$ ISCs[32], suggesting that CSCs may originate from Lgr5$^+$ ISCs in human colon adenocarcinoma. Gene mutation results in an imbalance between self-renewal and differentiation of ISCs, which is an important determinant for the formation of CRCs. Excessive ISC self-renewal expands the stem cell pool and promotes the

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total number of target cells transformed into cancer, thereby increasing the risk of tumorigenesis. In contrast, decreased self-renewal or enhanced differentiation, reduces the ISC population and impairs intestinal epithelial regeneration. Intestinal diseases, in turn, affect the function of ISCs and their TA cells. For example, inflammatory bowel disease is characterised by acute and chronic inflammation of intestinal tissue regions, and is accompanied by a decline of Paneth cell number[35]. The reduction of Paneth cell number damages the ISC niche, which reduces the expression of Lgr5 in crypt regions[35]. Lgr5$^+$ ISC loss has been found in the primary response to dextran sodium sulphate (DSS)-induced inflammation or high-dose γ-irradiation[36,37]. Recently, low density of Lgr5$^+$ ISCs and low differentiation toward enteroendocrine cells have been reported in patients with irritable bowel syndrome[38]. These data indicate that abnormalities in ISCs are key factors in the occurrence of intestinal diseases. Therefore, the precise regulation of ISC self-renewal and differentiation could provide new therapeutic opportunities for treating intestinal diseases.

Nutrient-sensing pathways in ISCs

It is a very important issue to understand how ISCs adjust their self-renewal and differentiation in response to diets and nutritional states to maintain intestinal homeostasis. Recent evidence shows that regulation of mTOR complex 1 (mTORC1) by nutrients, the Lkb1/AMPK pathway by energy, and the Sirt1 through nicotinamide adenine dinucleotide$^+$ (NAD$^+$) levels are involved in mediating the effects of dietary patterns on ISC function[3,11,39].

mTOR in ISCs

mTORC1 is a vital regulator that integrates multiple upstream signals, such as growth factors and nutrients to control cellular and organismal growth, protein synthesis, mitochondrial biogenesis and autophagy[40,41]. The mTORC1 pathway has been reported to be involved in ISC proliferation. In general, mTORC1 is abundantly expressed in Lgr5$^+$ ISCs but silenced in '+4' ISCs or TA cells. Upon exposure to damage, mTORC1 is activated in '+4' ISCs and further stimulates active Lgr5$^+$ ISCs to proliferate and differentiate into secretory cell progenitors and enterocyte progenitors[42,43]. Intestinal epithelium deletion of mTORC1 in mice results in atrophy of small intestinal villi and the defect of epithelial cells[44]. Interestingly, the '+4' ISC knockout of mTORC1 does not impact intestinal homeostasis under normal conditions, but impairs intestinal regeneration following injury[9]. These data indicate that mTORC1 in '+4' ISCs is mainly involved in the repair of damaged intestinal epithelium. Therefore, mTORC1 is required for intestinal epithelial regeneration, and elevated level of mTORC1 in '+4' ISCs is beneficial for intestinal health. However, aging-induced intestinal epithelial dysfunction is positively correlated with mTORC1 activity in ISCs or TA cells[39,45]. mTORC1 is activated in Lgr5$^+$ ISCs and Paneth cells during aging[45,46]. The activation of mTORC1 by tuberous sclerosis complex 1 deletion reduces organoid-forming capacity and the regenerative capacity of old intestinal epithelium[46]. In contrast, inhibition of mTORC1 partially rescues the regenerative capacity of aged intestinal epithelium in mice, which is attributable to the effects on both Paneth cells and ISCs[45,46]. Mechanistically, aging-induced mTORC1 activates the MKK6-p38-p53 pathway, resulting in exhaustion of ISC and decrease of villus size and density[45]. These data suggest that appropriate inhibition of mTORC1 in Lgr5$^+$ ISCs is beneficial for intestinal epithelial regeneration in the elderly.

Lkb1/AMPK in ISCs

Energy is an essential signal that influences ISC metabolism and homeostasis. AMPK, a master sensor of energy, is identified as a regulator of ISC proliferation[11]. AMPK can be activated in ISCs and crypts of mice in response to low-energy status, such as CR and fasting[11]. Inhibition of AMPK significantly inhibits the potential for high colony formation (proliferation) in ISCs of mice. On the contrary, activation of AMPK induces colony formation of ISCs[11], suggesting that AMPK is required for ISC proliferation. AMPK regulation of ISC function mainly depends on its downstream molecule Sirt1, which activates mTORC1 signalling to induce ISC expansion[11]. The tumour suppressor protein Lkb1 is identified as an upstream molecule of AMPK to control ISC fate. Lkb1 phosphorylation in ISCs is also significantly increased in CR mice, suggesting that AMPK activation in ISCs is likely attributable to the activation of Lkb1[11]. Lkb1 is also involved in the regulation of ISC fate in an AMPK-independent manner. Lgr5$^+$ ISC-specific Lkb1 deficiency in mice disrupts ISC homeostasis and promotes differentiation into secretory lineages by activation of Atoh1[47]. Lkb1 knockdown inhibits AMPK activity, but depletion of AMPKα1 or α2 of the catalytic subunit of AMPK fails to induce Atoh1 expression[47], indicating that AMPK is not required for the regulation of ISC fate by Lkb–Atoh1 axis.

Sirt1 in ISCs

Sirt1, an NAD-dependent protein deacetylase, plays important roles in cellular processes, including cell proliferation, differentiation and metabolism[48]. Recent evidence has implicated that Sirt1 acts as an important mediator in ISC proliferation in response to CR and aging[11,39]. Intestinal epithelium or Lgr5$^+$ ISC deletion of Sirt1 in mice blocks long-term CR-induced expansion of crypts and ISCs[11]. In contrast, Sirt1 overexpression in the intestine of mice mimics the role of CR, indicating that Sirt1 is required for the response to CR in the intestine. The activation of Sirt1 mainly depends on its upstream molecule AMPK, which leads to synthesis of NAD$^+$ by stimulating nicotinamide phosphoribosyl transferase (Nampt) in ISCs[49]. In addition, aging induces a decrease of Sirt1 level in ISCs and crypts, accompanied by a significant reduction in ISC number[39]. The activation of Sirt1 by supplementation with an NAD$^+$ precursor rescues the reduction in colony formation efficiency in crypt-derived organoids from old mice[39]. More importantly, the activation of Sirt1 also inhibits the increase in susceptibility to DSS in old mice. Therefore, regulation of ISC function by the Sirt1 may contribute to aspects of intestinal regeneration and protect from intestinal aging.

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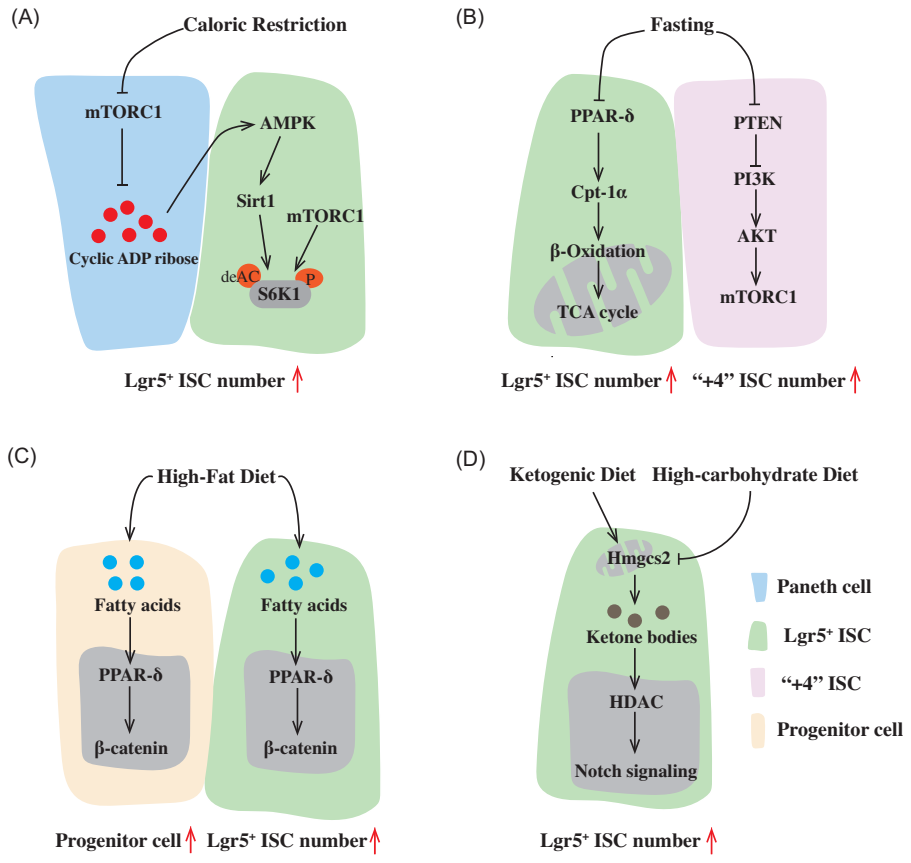

Fig. 2. Mechanisms of the effects of dietary patterns on ISC function. Dietary patterns, including caloric restriction, fasting, high-fat diet, ketogenic diet and high-carbohydrate diet, control ISC homeostasis through nutrient-sensing pathways. (A) Caloric restriction inhibits mTORC1 signalling in Paneth cells, and further increases the level of cyclic ADP ribose (cADPR). Paneth cell-derived cADPR induces the activation of mTORC1 in ISCs via the AMPK–Sirt1 axis and increases Lgr5$^+$ ISC number, thereby promoting intestinal regeneration. (B) Fasting induces PPAR-δ-mediated fatty acid β-oxidation and improves ISC number, and prevents aging induced-intestinal injury. Fasting also inhibits phosphatase and tensin homolog (PTEN), a phosphatase that negatively regulates PI3K-AKT-mTOR signalling, and increases '+4' ISC number and contributes to intestinal regeneration. (C) High-fat diet increases the number and self-renewal of ISCs, enables ISCs to become niche independent, and confers stemness to non-Lgr5$^+$ progenitors through PPAR-δ signalling, which contributes to intestinal tumorigenesis. (D) Ketogenic diet improves ketone bodies levels in Lgr5$^+$ ISCs, leading to higher Notch activity and ISC number, and promotes post-injury regeneration; compared with ketogenic diet, high-carbohydrate diet has opposite effects.

Dietary patterns and ISC function

Caloric restriction

CR is defined as energy intake which is 60–80 % of normal caloric intake, without causing malnutrition[50]. CR has been reported to maintain intestinal epithelial homeostasis through regulating ISC proliferation (Fig. 2A)[10]. The number of ISCs is significantly increased in CR-treated mice[51]. Interestingly, co-cultivation of Paneth cells from CR mice and ISCs from *ad libitum* mice also promotes ISC proliferation[51]. This indicates that regulation of Paneth cell niche by CR controls ISC function. Mechanistically, CR inhibits mTORC1 signalling in Paneth cells, and further increases the level of cyclic ADP ribose (cADPR)[51]. Paneth cell-derived cADPR induces the activation of mTORC1 in ISCs via AMPK–Nampt-Sirt1 axis, which promotes ISC proliferation (Fig. 2A)[11]. CR also increases the pool of '+4' ISCs and has a beneficial effect on the regenerative capacity of intestinal epithelium in response to damage. As opposed to Lgr5$^+$ ISCs, CR downregulates mTORC1 signalling in '+4' ISCs[9]. This seems to be a paradox between the increase in '+4' ISC number and the decrease in mTORC1 signalling, because mTORC1 signalling activation promotes cell proliferation. In fact, ISCs with high expression of mTORC1 are more sensitive to undergo apoptosis, and few mTORC1-high expressed cells remain present in '+4' ISCs upon CR[9]. Upon exposure to radiation injury, more mTORC1-low expressed ISCs are activated to contribute to epithelial regenerative capacity[9]. Due to the unique roles of mTORC1 in Lgr5$^+$ ISCs and '+4' ISCs, regulation of intestinal epithelial regeneration with mTORC1 as a target will face a difficult challenge.

Fasting

Fasting, which is also called food deprivation, has been shown to be effective in increasing lifespan and promoting tissue regeneration by improving adult stem cell function in diverse tissues[52,53]. Growing evidence has shown that fasting promotes intestinal epithelial regenerative capacity after damage through the preservation of ISC function[7,10,12,13,54]. Fasting for 48 h inhibits phosphatase and tensin homolog, a phosphatase that negatively regulates PI3K–AKT–mTOR signalling, and increases '+4' ISC number and contributes to intestinal regeneration

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(Fig. 2B)[13]. A fasting-mimicking diet (50 % of standard daily calorie intake on day 1, and 10 % of standard daily calorie intake on days 2–4) ameliorates DSS-induced inflammatory bowel disease by promoting ISC activity[7]. Tinkum *et al.* reported that 24 h fasting protects against small-intestinal injury by boosting the regenerative potential of ISCs undergoing dose-intensive chemotherapy[12]. Similarly, 24 h fasting not only increases surviving crypt number, but also promotes the organoid-forming capacity in ISCs and crypts[10]. Different with the CR phenotype[11,51], co-culture of ISCs with fasting Paneth cells does not increase the ability of organoid formation, suggesting that fasting regulates ISC function directly. In addition, fasting also induces PPAR-δ-mediated fatty acid β-oxidation and reverses the age-dependent decline of ISC number and function, and improves intestinal repair after injury in the elderly (Fig. 2B)[10]. Collectively, the beneficial effect of CR and fasting on intestinal function has been extensively confirmed in animal models. Further clinical trials are needed to evaluate their roles in human intestinal health.

High-fat diet

Excess caloric intake induces obesity and is related to cancer incidence in many tissues, especially in the gastrointestinal tract. Long-term HFD (60 % fat, 9–14 months) feeding mildly reduces intestinal villi height and villus enterocyte numbers, and increases crypt depth in mice[3,55]. However, short-term HFD (45–60 % fat, 8–20 weeks) feeding increases both villi height and crypt density[56–58]. This observation indicates that short-term HFD feeding may not be long enough to induce the effects on intestinal morphology observed with longer HFD feeding. Consistently, both long-term and short-term HFD feeding increases the number and proliferation of ISCs and progenitor cells and reduces Paneth cell number, which contributes to intestinal tumour formation (Fig. 2C)[3,55–57]. *Ex vivo* experiments have confirmed that ISCs and crypts from HFD-fed mice exhibit fewer crypt budding (a process of both ISC self-renewal and differentiation) domains and reduce organoid-forming ability[3,56]. Reciprocally, CR also increases the number of ISCs, but decreases the risk of intestinal tumour formation[59]. The mechanistic differences underlying altered ISC function in the two dietary patterns may be the main reason for this discrepancy. Mechanistically, HFD-induced PPAR-δ activation not only increases ISC number via the Wnt/β-catenin pathway, but also enhances the ISC-like progenitor population to induce intestinal tumorigenesis[3]. HFD-induced obesity also enhances plasma insulin and insulin-like growth factor 1 (IGF-1) levels to impact on ISC hyper-proliferation[56]. Andres *et al.* have reported that deletion of insulin receptor in intestinal epithelial cell can block the HFD-induced increase in ISC marker expression[55]. These data suggest that insulin signalling is required for HFD-induced ISC proliferation. Therefore, reducing fat intake may be a potential intervention strategy to prevent intestinal tumorigenesis in humans.

Ketogenic diet

Ketogenic diet is characterised by high fat, moderate protein and low carbohydrate, which dramatically elevates circulating ketone body levels[60]. Recent evidence implicates an emerging role for ketone bodies in regulating Lgr5$^+$ ISC function and intestinal epithelial homeostasis (Fig. 2D)[8,61]. Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase 2 (Hmgcs2), a rate-limiting enzyme for ketone bodies synthesis, is selectively expressed in ISCs[8,62]. Deficiency of Hmgcs2 in Lgr5$^+$ ISCs damages ISC stemness and induces their differentiation into Paneth and goblet cells by increasing HDAC-mediated suppression of the Notch signalling[8]. The ketogenic diet increases ketone body concentrations both systemically and within ISCs and boosts ISC number and function to improve intestinal regenerative capacity[8]. Wang *et al.* have reported that a ketogenic diet significantly increases Hmgcs2 expression and promotes intestinal epithelial cell differentiation to maintain intestinal homeostasis[63]. Therefore, ketogenic diet may act as a dietary intervention for treating intestinal diseases.

High-carbohydrate diet

High-carbohydrate-based dietary models such as the high-sugar diet and the Western diet have deleterious consequences for metabolic health. Excessive high-sugar diet intake increases blood glucose, insulin and triacylglycerol levels, which contributes to the prevalence of obesity and type 2 diabetes[64]. Recent studies have shown that high-carbohydrate diet damages intestinal epithelial regenerative capacity through the regulation of ISC function. A glucose-supplemented diet (13 % glucose in drinking-water) diminishes the self-renewal capacity of ISCs and promotes their differentiation towards Paneth and goblet cells[8]. These processes suppress intestinal ketogenesis and ISC stemness, and damage post-injury regeneration[8]. In *Drosophila*, a high-sugar diet promotes ISC differentiation through up-regulation of the JNK pathway and down-regulation of the JAK/STAT pathway, and induces disruption of intestinal homeostasis[65]. Together, these data suggest high-carbohydrate diet dampens the number and regenerative capacity of ISCs, and is harmful for intestinal homeostasis.

Nutrients and ISC function

Amino acids

Amino acids are essential nutrients for maintaining intestinal epithelial homeostasis and preventing intestinal diseases. Methionine is an important essential amino acid that promotes ISC self-renewal (Table 1)[18,66]. *Ex vivo* experiment has observed that methionine deprivation leads to a decrease in ISC proliferation, and enhances ISC differentiation toward secretory cells, including goblet, enteroendocrine and Paneth cells[18]. Evidence also confirmed that depletion of dietary methionine inhibits ISC proliferation in *Drosophila*[66]. *S*-adenosylmethionine, a universal methyl donor from methionine metabolism, is a critical metabolite for maintaining ISC proliferation via the regulation of methyltransferases[66,67]. Arginine is also proven to be an indispensable nutrient for ISC proliferation and intestinal epithelial renewal (Table 1)[68]. Arginine deficiency inhibits mouse organoid survival and growth and is accompanied by the loss of Paneth cells and Lgr5$^+$ ISCs. The potential mechanism is that arginine can induce Wnt3a production by Paneth cells,

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Table 1. Effects of nutrients on ISC self-renewal and differentiation

Nutrients	Category	Phenotype	Refs
Methionine	A neutral amino acid	Methionine deficiency inhibits ISC proliferation, and enhances ISC differentiation toward secretory cells in mouse organoids	(18)
		Dietary methionine deficiency inhibits ISC proliferation in *Drosophila*	(64)
Arginine	A basic amino acid	Arginine deficiency leads to a decrease in number of Paneth cells and Lgr5$^+$ ISCs, and inhibits mouse organoid survival and growth	(68)
Glutamine	A neutral amino acid	Glutamine promotes crypt expansion and ISC proliferation in mouse jejunal organoids	(70)
Glutamate	An acidic amino acid	Glutamate enhances ISC proliferation and organoid formation in pig and *Drosophila*	(69, 71)
Palmitate	A saturated fatty acid	Palmitate increases Lgr5$^+$ ISC number and gives rise to more secondary organoids in mouse	(10)
Oleic acid	A monounsaturated fatty acid	Oleic acid increases Lgr5$^+$ ISC number in mouse and human intestinal organoids	(3)
Arachidonic acid	An essential fatty acid	Arachidonic acid promotes the proliferation of '+4' ISCs, and inhibits differentiation of small intestinal organoids in mouse	(16)
Vitamin D_3	A fat-soluble vitamin	Dietary vitamin D_3 deficiency reduces Lgr5$^+$ ISC number and inhibits their proliferation activity in mouse	(19)
Vitamin A	A fat-soluble vitamin	Vitamin A increase Lgr5$^+$ ISC self-renewal and inhibits ISC differentiation in porcine small intestinal organoids	(75)
Bile acid	A gut microbial metabolite	Bile acid promotes expansion of the Lgr5$^+$ ISC pool and induces ISC self-renewal in mouse	(17)
Butyrate	A gut microbial metabolite	Butyrate inhibits the proliferation of colonic Lgr5$^+$ ISCs or precursor cells in mouse	(82)
Lactate	A gut microbial metabolite	Lactate increases the number of Lgr5$^+$ ISCs and small intestinal organoid size in mouse	(20)
Indole-3-carbinol	A tryptophan metabolite	Indole-3-carbinol inhibits Lgr5$^+$ ISC number and intestinal organoid formation, and promotes the formation of goblet and Paneth cells in mouse	(85)
Indole-3-aldehyde	A tryptophan metabolite	Indole 3-aldehyde increases the proliferation of ISC and Paneth cell populations in mouse	(80)

which provides the niche factors for ISC self-renewal[68]. Glutamine and glutamate have also been reported to impact ISC self-renewal and differentiation (Table 1)[69–71]. Medium supplemented with glutamine significantly promotes crypt expansion via the activation of mTORC1 signalling in mouse jejunal organoids[70]. Glutamine deficiency inhibits organoid formation and induces crypt atrophy[70]. Additionally, dietary glutamate enhances organoid formation and budding efficiency as well as ISC proliferation in pig and *Drosophila*[69,71]. We recently found that aspartate can alleviate DSS-induced intestinal epithelial damage by activating '+4' ISCs, while reducing the number of Lgr5$^+$ ISCs (unpublished results). However, the exact mechanisms underlying the autonomous regulation of ISC function by aspartate remain to be identified.

Fatty acids

The effects of the HFD pattern on villus height and crypt depth suggest that fatty acids may play a crucial role in ISC function. Fatty acids, especially long-chain fatty acids, have been reported to influence ISC proliferation and organoid formation (Table 1). Treatment of mouse organoids with palmitate and/or oleic acid boost Lgr5$^+$ ISC number and give rise to more secondary organoids[3,10]. Consistent with HFD studies, β-oxidation controls fatty-acid-driven ISC self-renewal[3]. In metabolic flux studies, $^{13}C_{16}$-palmitate is efficiently incorporated into citrate and α-ketoglutarate in crypts, with over 20 % of carbons labelled with ^{13}C[10,72]. These data suggest that palmitate is oxidised to acetyl-CoA and enters into the TCA cycle for combustion. Indeed, acetate, an alternate source of acetyl-CoA, completely rescues the growth and budding defects and significantly increases organoid formation by inhibition of β-oxidation[72,73]. However, acetate does not affect the growth, budding or passaging capacity of common organoids, suggesting that acetate is required for ISC self-renewal only when acetyl-CoA concentration is reduced. Recent study has reported that arachidonic acid promotes the proliferation of '+4' ISCs by activating Wnt signalling, and facilitates small-intestinal regeneration (Table 1)[16]. In summary, fatty acids may serve as nutrient substrates to promote ISC proliferation.

Vitamins

Vitamin D, a highly pleiotropic hormone, is an essential nutrient that maintains Lgr5$^+$ ISC proliferation (Table 1). Deficiency of dietary vitamin D_3 reduces Lgr5$^+$ ISC number and their proliferation activity in mice[19]. In addition, a low level of dietary vitamin D_3 increases enterocyte number, and decreases the number of secretory cells accompanied by a prominent increase in ectopic expression of Paneth cell markers[19]. More importantly, long-term feeding of a low-vitamin D_3 diet induces sporadic intestinal tumour formation in mice, but the pro-tumour effects can be prevented by supplementing dietary vitamin D_3[19]. ISC-specific knockout of the vitamin D receptor also decreases the proliferation ability of Lgr5$^+$ ISCs at the bottom of the crypt[19]. Activation of the vitamin D receptor downregulates the β-catenin target genes c-MYC and PPAR-δ, which may mediate the anti-oncogenic effects of vitamin D signalling[74]. Collectively, vitamin D is indispensable for maintaining proliferative ability of Lgr5$^+$ ISCs via the vitamin D receptor signalling pathway. Vitamin A also has been reported to maintain intestinal epithelial renewal and development (Table 1). Dietary supplementation of vitamin A promotes villus height and crypt depth and Lgr5 expression in the jejunum mucosa of piglets[75]. Treatment with vitamin A metabolites retinol and retinoic acid significantly reduces the budding rate and budding number of

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organoids cultured from piglet small intestine[75]. Moreover, the mRNA expression of stem cell markers is increased and chromogranin A and muc2 expression is decreased, suggesting that vitamin A may increase ISC self-renewal at expense of differentiation[75].

Bile acids

Bile acids are synthesised from cholesterol by hepatocytes and participate in emulsification and absorption of dietary lipids[76]. Bile acids are recently attracting extensive attention as a vital modulator of intestinal health and disease[77]. Bile acids promote intestinal epithelial regeneration via promoting ISC self-renewal in response to injury (Table 1)[17]. Mechanistically, bile acids can activate G-protein-coupled bile acid receptor 1, promoting expansion of the $Lgr5^+$ ISC pool by the activation of SRC/YAP signalling[17]. Interestingly, evidence has revealed that bile acids act as potent factors for CRC owing to the promotion of CSC number[78]. HFD induces the increase of intestinal bile acids to promote CSC proliferation, thereby contributing to the initiation of CRC[79]. Secondary bile acids, including deoxycholic acid and lithocholic acid, also induce the expansion of CSCs via Wnt/β-catenin signalling, and thus lead to CRC formation[78]. In short, bile acids likely act as a double-edged sword for intestinal health, and not only promote intestinal epithelial regeneration but also induce CRC formation via different mechanisms.

Gut microbial metabolites

The interactions between microbial metabolites and ISC homeostasis have been widely studied in recent years. Probiotics, including *Bifidobacterium* and *Lactobacillus spp.,* have the promotive roles in ISC proliferation and organoid formation either under physiological or pathological conditions[20,80,81]. The effects of intestinal microbiota on ISC function depend on their microbial metabolites. These metabolites include lactate, short-chain fatty acids and tryptophan metabolites (Table 1). Lactate is sensed by its receptor GPR81 on Paneth and stromal cells to promote $Lgr5^+$ ISC proliferation by activating the Wnt3/β-catenin signalling pathway[20]. Butyrate inhibits the proliferation of colonic ISCs by inhibiting HDAC activity[82]. However, butyrate does not affect the $Lgr5^+$ ISC pool and budding efficiency in small intestinal organoids[83]. The unique role of butyrate highlights its important position in maintaining colonic epithelial homeostasis. Tryptophan metabolites, including indole, indole-3-carbinol and indole-3-aldehyde, impact ISC proliferation via aryl hydrocarbon receptor or pregnane X receptor signalling. For example, dietary supplementation with indole-3-carbinol restricts $Lgr5^+$ ISC proliferation via suppression of Wnt3/β-catenin signalling and prevents tumorigenesis[84,85]. Indole-3-aldehyde increases the number of ISCs via activation of STAT3 phosphorylation, and promotes post-injury regeneration[80]. At present, the mechanisms by which microbial metabolites regulate ISC homeostasis are still poorly understood because of the complexity of microbiota. Future studies revealing mechanistic insights on regulation of ISC function by microbial metabolites will hopefully provide novel strategies to combat intestinal diseases.

Concluding remarks and future perspectives

Diet has an important influence on intestinal health and cancer incidence. Based on data from current literature, CR and fasting are generally beneficial for intestinal health, such as through inducing ISC self-renewal to promote intestinal regeneration after injury and reversing aging-related intestinal dysfunction. Excessive fat intake is harmful to intestinal health because it can expand the pool of ISCs and induce tumorigenesis. The ketogenic diet is associated with ISC self-renewal, and may become the most promising nutritional intervention strategy for treating intestinal diseases. High-carbohydrate diet disrupts intestinal homeostasis through the inhibition of ISC self-renewal. Nutrient-sensing pathways, including mTORC1, Lkb1/AMPK and Sirt1, are involved in regulation of both beneficial and harmful roles of dietary patterns in ISC function and cancer initiation. Nutrients such as amino acids, fatty acids, vitamins and gut microbiota metabolites directly control ISC self-renewal and differentiation to maintain intestinal epithelial homeostasis via their distinct signalling pathways. However, studies of individual nutrients in ISC function are still lacking. Further research is needed to elucidate the effects of various nutrients on ISC homeostasis and their regulatory mechanisms. Finally, extensive additional work will be required to elucidate ISC function in large animals, such as pigs with intestinal physiology and feeding behaviour similar to human[86], which will provide more valuable insights for prevention of human intestinal diseases.

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