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Roles of intestinal stem cells in inflammatory bowel disease pathogenesis

Zhang MJ *et al*. Intestinal stem cells in IBD

Abstract

Inflammatory bowel disease (IBD), consisting primarily of ulcerative colitis and Crohn's disease, is a chronic, relapsing inflammatory disorder of the gastrointestinal tract. The pathogenesis of IBD has been thoroughly studied throughout the past few decades, such as defective gut epithelial barrier, immune responses, genetic predisposition, infections, and dysbiosis. Recent studies have revealed the unexpected importance of intestinal stem cells (ISCs) in the pathophysiology of IBD. The rapid recovery and continuous self-renewal of intestinal epithelial cells depend on ISCs within the crypts. Proliferation and differentiation of ISCs is an important cytological basis for repairing damaged intestinal mucosa. Unfortunately, as a new therapeutic goal in IBD, mucosal healing is difficult to achieve with current treatments. Stem cell therapy is an emerging treatment for IBD that allows mucosal healing by rebuilding the mucosal barrier. In this review, we present the current research progress on the role of ISCs in IBD and discuss stem cell-based therapies that have been specifically designed for its treatment.

Key Words: Intestinal stem cells; Inflammatory bowel disease; Pathogenesis; Therapy potential; Clinical application

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Core Tip: Inflammatory bowel disease (IBD) is a chronic and relapsing inflammatory disorder of the gastrointestinal tract that severely compromises the quality of life of patients. Intestinal stem cell proliferation and differentiation underlie damaged mucosa repair. However, mucosal healing - a key IBD therapeutic target - remains elusive with current treatments. Emerging stem cell therapy promotes mucosal barrier restoration, offering IBD intervention. This review examines intestinal stem cell roles in IBD pathogenesis and discusses specifically engineered stem cell-based therapies for management.

INTRODUCTION

Inflammatory bowel disease (IBD) is a group of recurrent, chronic, non-specific inflammatory diseases of the bowel, including Crohn's disease (CD) and ulcerative colitis (UC)[1,2]. An increasing number of patients are found not only in Western countries but also in newly industrialized regions such as Asia[3]. IBD is clinically characterized by diarrhea, mucous and bloody stools, abdominal pain. Many extraintestinal manifestations occur in patients with IBD commonly involve the musculoskeletal system, skin, hepatobiliary tract and eyes[4]. The pathogenesis of IBD is not fully understood and involves factors such as complex genetic, environmental, epithelial, microbial, and mucosal immunity[5,6]. The breakdown of the intestinal barrier, allowing microorganisms and other antigens to penetrate the bowel wall and trigger uncontrolled immune activation, is a hallmark of IBD[7-9].

In mammals, the intestine is covered by a single layer of epithelial cells that is renewed every 96-120 hours[10]. Intestinal stem cells (ISCs) are important adult stem cells mainly located in intestinal crypts, which continuously differentiate and regenerate themselves to achieve intestinal epithelial cell renewal[11,12]. Within the crypt, constantly dividing stem cells give rise to progenitor cells (or transit-amplifying cells), which rapidly proliferate before differentiating into mature intestinal epithelial cells. These cells then continue to divide, giving rise to the two differentiated lineages present in the villus: Secretory cells and absorptive cells[13,14]. The two intestinal epithelial lineages underpin the primary physiological functions of the gastrointestinal tract. The secretory lineage includes four distinct cell types - mucus-secreting goblet cells, hormone-producing enteroendocrine cells (EECs), antimicrobial peptide-releasing Paneth cells (PCs) and immune regulating-tuft cells - that primarily function in maintaining the integrity of the epithelium[12]. In contrast, the absorptive lineage consists of enterocytes, which are responsible for nutrient absorption and represent the most prevalent cell type in the intestine[15]. Tissue homeostasis is dependent on the ISC niche[12].

Approaches to managing IBD are shifting from simply controlling symptoms to aiming for mucosal healing, with the goal of preventing disease progression and avoiding bowel damage[16,17]. Currently approved drugs for IBD include corticosteroids, thiopurine, methotrexate biologics and tiny chemical compounds[18-20]. Although new biologics and small molecule drugs have demonstrated promising efficacy, they are not without potential side effects, such as immunosuppression, heightened infection risks, and possible cancer concerns[21]. Meanwhile, a significant number of patients do not respond to these biologics, underscoring the need for alternative approaches to achieve mucosal healing in IBD. For patients who are unresponsive to biologics, stem cell-based therapies may represent a promising new approach for restoring the epithelial barrier in IBD. In the past two decades, studies have demonstrated that the transplantation or transfusion of mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs) can offer beneficial effects for IBD patients[22,23]. Meanwhile, advancements in ISC sorting technology and the refinement of culture systems have led to substantial progress in understanding the biological characteristics of ISCs. This has, in turn, facilitated the clinical translation of ISC-based therapies for gastrointestinal disorders. This review outlines the current research on the role of ISCs in IBD and investigates stem cell-based strategies for clinical application.

OVERVIEW OF ISCS

Types and marks of ISCs

Earlier discussions on ISC identity primarily centered around their physical location within the intestinal crypt. As early as 1974, it was suggested that the crypt base columnar cells (CBCs) might serve as the progenitor cells of the intestinal epithelium by Cheng and Leblond[24]. In a series of electron microscopy studies on small intestinal crypts, Cheng and Leblond[24] observed slender, immature, cycling cells situated between PCs at positions 1-4 of the crypt base with proliferation occurring roughly once every day. The absence of specific molecular markers to identify these cells has prevented enough definitive evidence to confirm that CBC cells are stem cells. In 2007,

lineage-tracing experiments in adult mice were carried out using an inducible Cre knock-in allele and the Rosa26-lacZ reporter strain. Over a 60-day period, leucine-rich repeat-containing G protein-coupled receptor 5 (Lgr5)-positive CBCs generated all epithelial lineages, providing evidence that they function as the stem cells of the small intestine and colon[25]. In 2009, through a lineage tracing approach, Sato *et al*[26] demonstrated that *ex vivo* cultured Lgr5+ CBCs are capable of generating all intestinal epithelial lineages. Therefore, LGR5+ CBC cells satisfy both key criteria of stemness: The capacity to generate multiple lineages and maintain long-term self-renewal. Additionally, lineage tracing studies revealed that certain Lgr5+ cells also express prominin-1 (CD133), and these CD133+ cells have the capacity to give rise to the entire intestinal epithelium[27]. In recent years, additional potential markers of CBCs have been identified including olfactomedin-4 (Olfm4)[28], achaete-scute homolog 2[29] and SRY-related high-mobility group box 9[30], in addition to Lgr5 and CD133.

In addition to CBCs, a unique population of quiescent "reserve" ISCs is found at the fourth position from the bottom of the crypt, just above the differentiated PCs. These cells, termed +4 cells, were first identified by Potten[31] and Potten *et al*[32]. Due to the limitations of contemporary technology, the stemness of this population could not be easily established, prompting several research teams to focus on identifying specific genetic markers. Several studies have since proposed alternative ISC markers, which are primarily enriched in the +4 cell population. Sangiorgi and Capecchi[33] identified that B cell-specific Moloney murine leukemia virus insertion site 1 (Bmi1) is expressed in a specific population of cells located near the crypt base in the small intestine, primarily at the +4 position, four cells above the base. These cells proliferate, expand, renew, and ultimately give rise to all the differentiated cell lineages of the small intestinal epithelium. In 2011, Takeda *et al*[34] showed that homeodomain only protein (Hopx), an atypical homeobox protein, serves as a specific marker for +4 cells. Hopx-expressing cells give rise to CBCs and all mature intestinal epithelial lineages, while CBCs can also differentiate into +4 Hopx-positive cells. These findings establish a bidirectional lineage relationship between active and quiescent stem cells within their

niches. In addition to Bmi1 and Hopx, other +4 cells markers have been identified in the last years, including telomerase reverse transcriptase[35] and leucine-rich repeats and immunoglobulin-like domains containing protein 1[36]. Subsequent comprehensive expression analyses, employing single-molecule fluorescent *in situ* hybridization, transcriptomics, and proteomics, revealed that although these markers are predominantly enriched in +4 cells, they are also likely expressed in other crypt cell populations, including CBCs[37-39]. The +4 stem cell is now regarded as a reserve stem cell with high resistance to radiation, capable of replenishing the pool of continuously cycling CBC cells when necessary[40]. The question of whether +4 cells and CBCs represent two distinct ISC populations, and whether this distinction is an intrinsic property or a consequence of their different locations within the ISC niche, remains a topic of ongoing debate[41].

Lgr5+ cells have been widely accepted as the model for the only homeostatic ISCs supporting intestinal epithelial regeneration. But recently, Malagola *et al*[42] found that stemness potential extends beyond the crypt base, residing in the isthmus region, where undifferentiated cells contribute to intestinal homeostasis (Figure 1). By using a kinetic reporter for time-resolved fate mapping and fibroblast growth factor binding protein 1 (Fgfbp1)-CreER^{T2} lineage tracing, Capdevila *et al*[43] demonstrate that Fgfbp1+ cells in upper crypt are multipotent and can give rise to Lgr5+ cells, supporting their role as ISCs. Moreover, Fgfbp1+ cells are capable of sustaining epithelial regeneration following the depletion of Lgr5+ cells. Intestinal epithelial regeneration originates from the upper crypt rather than the crypt base, challenging previous understanding.

ISC niche

ISCs are sustained by the surrounding niche cells, which play a crucial role in maintaining their proliferative potential and self-renewal capacity (Figure 1). At the base of the crypt, ISCs are encased by a heterogeneous population of stromal cells, including pericryptal myofibroblasts, fibroblasts, endothelial cells, neural cells, immune cells, pericytes, and smooth muscle cells. In concert with other niche components such

as PCs and EECs, these stromal cells modulate the proliferative activity of ISCs, the differentiation of mature intestinal epithelial cells, and their overall survival[44]. And the colon possesses a crypt-based ISC niche similar to that of the small intestine but lacks PCs. PCs produce antimicrobial substances, such as α-defensins, lysozyme, and phospholipase A2, along with the Wnt activator protein Wnt3 and transforming growth factor-alpha (TGF-α), which are essential for safeguarding ISC proliferation[45,46]. EECs, primarily enterochromaffin cells, have the ability to dedifferentiate into fully functional ISCs through asymmetric cell division and may also serve as a reserve pool of ISCs[47]. Stromal cells also constitute an integral component of the niche. *In situ* hybridization studies have demonstrated that several Wnt ligands, including Wnt-2b, Wnt-4, Wnt-5a, and Wnt-5b, as well as R-spondins (RSPOs), are expressed within the stromal compartment to support the function of ISCs[48]. Twist2+ stromal cells have recently been identified as a niche subpopulation that supports canonical ISCs by secreting Wnt ligands[49]. Immune cells are mainly located in the lamina propria, directly beneath the crypts[50]. Recent advances in organoid culture and single-cell sequencing have shown that intestinal immune cells not only mediate immune responses through cytokine release, but also function as key components of the ISC niche, regulating stem cell fate to maintain epithelial homeostasis and drive regeneration during tissue repair[51].

Signaling pathways that regulate ISC niche functions

ISCs are regulated by multiple developmental signaling pathways, including Wnt, epidermal growth factor (EGF), Notch and bone morphogenetic protein (BMP), which are crucial for maintaining the balance between enterocyte proliferation, maturation, and migration, and play pivotal roles in governing the fate of ISCs (Figure 2). Below, we underscore the pivotal role of several key signaling pathways, coordinated between the epithelium and mesenchyme, in determining the identity and function of ISCs.

Wnt: The canonical Wnt/β-catenin signaling pathway serves as a crucial regulator of ISC proliferation. Wnt ligands comprise a family of 19 secreted glycoproteins that exert their signaling effects by binding to Frizzled receptors and lipoprotein receptor-related protein 5/6 coreceptors. They transduce the signal either *via* β-catenin in the canonical pathway or through a series of other proteins in the non-canonical pathway. Extracellular Wnt signaling triggers multiple intracellular signaling cascades, including the Wnt/β-catenin-dependent (canonical) pathway and the β-catenin-independent (non-canonical) pathway, the latter of which can be further subdivided into the planar cell polarity pathway and the Wnt/Ca^{2+} signaling pathway[52].

A hallmark of the Wnt/β-catenin canonical pathway is the accumulation and translocation of the adhesion-related protein β-catenin to the cell nucleus, where it binds to T-cell factor (TCF) family of transcription factors and directly regulates gene expression[53,54]. Systemic knockout of *Tcf4* leads to the complete depletion of ISCs, followed by the disruption of the epithelial structure in neonatal mice[55]. Moreover, the deletion of *Tcf7 L2* results in the loss of Lgr5+ ISCs, underscoring the essential role of this pathway in adult intestinal regeneration[56]. On the other hand, a finely tuned balance between Wnt agonists and antagonists within the ISC niche is crucial for preserving the integrity of the ISC compartment. Disruption of this balance, often caused by mutations in the tumor suppressor gene adenomatous polyposis coli results in uncontrolled activation of the Wnt signaling pathway[57]. Adenomatous polyposis coli-mutant ISCs act as bona fide supercompetitors by secreting Wnt antagonists, thereby promoting the differentiation of neighboring wild-type ISCs[58]. The epithelial Wnt ligand is secreted by PCs as Wnt3[45]. As ISCs begin to differentiate and exit the stem cell zone, the level of Wnt3 gradually diminishes. This generates a Wnt3 concentration gradient along the crypt-villus axis, thereby establishing a dynamic negative feedback loop[59]. In addition, the activation of the Wnt pathway relies on RSPOs, which are secreted by mesenchymal cells to enhance Wnt activity in the crypt[60]. RSPOs, a family of four secreted soluble proteins, bind to LGR family receptors, thereby enhancing the stability of Frizzled receptors[61,62]. RSPO2 and

RSPO3 are essential for the maintenance of LGR5+ stem cells in the intestinal crypt, as neutralizing antibodies against both RSPOs are sufficient to cause the depletion of these cells[63]. Furthermore, the growth and maintenance of ISCs depend on lymphatic endothelial cells and RSPO3+GREM1+ epithelial cells. In the ISC niche, these two cell types are in close proximity and exert their function through the secretion of RSPO3[64].

EGF: In organoid culture models, EGF, a key component secreted by PCs and essential for establishing *ex vivo* cultures, regulates organoid growth and differentiation toward the EEC lineage, and is a crucial part of the intestinal organoid culture medium[45,65]. Inhibiting EGF signaling in intestinal organoids causes proliferative LGR5+ ISCs to enter quiescence and halts organoid growth[65]. Additionally, the interleukin (IL)-33/suppression of tumorigenicity 2 axis, an immunomodulatory pathway that is clinically monitored as a biomarker for intestinal injury, controls intrinsic epithelial regeneration by stimulating the production of EGF[66]. In Drosophila ISCs, EGF signaling activates ISCs by enhancing mitochondrial biogenesis and β-oxidation[67]. It is important to highlight that neuregulin 1 (NRG1), a key ligand of the EGF family, plays a pivotal role in tissue repair following injury. In contrast to EGF, NRG1 is upregulated in response to damage and is expressed in mesenchymal stromal cells, macrophages, and PCs. The absence of NRG1 impairs proliferation in intestinal crypts and dimini. lshes regenerative potential[68]. These results highlight the important roles of EGF ligands in ISC regulation.

Notch: The Notch signaling pathway is one of the most important pathways in determining cell fate. The canonical Notch signaling is initiated through the interaction between Notch ligands on neighboring cells and the Notch receptor, resulting in a series of proteolytic cleavages. This releases the Notch intracellular domain into the cytoplasm, from where it translocates to the nucleus. There, Notch intracellular domain binds to the DNA-binding transcription factor cellulose synthase-like, forming a complex that activates the transcription of downstream genes[69]. Notch receptor and

ligand mRNAs have been identified in both epithelial and mesenchymal cells[70]. Notch signaling regulates the differentiation of CBCs into either absorptive or secretory cells. Activation of Notch promotes differentiation towards absorptive cells, whereas inhibition of Notch favors differentiation into secretory cells. Notch signaling is essential for maintaining the ISC pool, and its inhibition results in a reduction of Lgr5+ cell numbers and impaired proliferation[70]. Expression of Notch target genes in intestinal crypts, with hairy and enhancer of split 1 (Hes1) and Olfm4 localized to CBC cells[71,72]. PCs, located adjacent to CBC stem cells at the crypt base, express the Notch ligand delta-like ligand 4 (DLL4). The expression of genes known to support ISC function (Dll4, Wnt3, EGF) in PCs suggests that these cells may act as niche cells for ISCs[73]. Deletion of either the Notch ligands (Dll1 and Dll4) or the Notch effectors (Hes1, Hes3, and Hes5) leads to the loss of crypt proliferation[72,74]. Atonal homolog 1 (Atoh1) is a key transcriptional activator that fully drives the differentiation of secretory cells. Inactivation of Notch in progenitor cells leads to the upregulation of Atoh1 expression, driving differentiation towards the secretory lineage[75]. And intestinal epithelial *Atoh1* knockout results in the loss of the entire secretory lineage without affecting absorptive cell differentiation[76]. Furthermore, a recent study demonstrates that mitochondrial dynamics, governed by FOXO and Notch signaling, are essential in driving stem cell differentiation into secretory cell types, including goblet cells and PCs[77].

BMP: BMPs are members of the TGF-β superfamily. In mammals, over 12 BMP-related proteins have been identified, including BMPs 2, 4-10, osteogenin-1, and growth differentiation factors 5-7, among others[78]. The BMP signaling pathway serves to antagonize the proliferative signals within the ISC niche, thereby inhibiting stem cell self-renewal and driving cellular differentiation. Inhibition of BMP signaling impairs the terminal differentiation of the secretory lineage, resulting in dysregulated goblet cell maturation within the intestinal epithelium[79]. In addition, different mesenchymal cell populations generate a crucial intestinal BMP signaling gradient. Platelet-derived

growth factor alpha (high) telocytes, abundant at the villus base, serve as a BMP reservoir, while a CD81+ platelet-derived growth factor alpha (low) population located just beneath the crypts secretes the BMP antagonist Gremlin1[80]. These cells can expand ISCs *in vitro* without additional nutritional support and contribute to ISC maintenance *in vivo*[80]. Dysfunction of BMP signaling in stromal cells leads to excessive proliferation of mesenchymal cells and the excessive secretion of interleukins, which further promote abnormal goblet cell differentiation and excessive synthesis and accumulation of mucus, resulting in structural and functional abnormalities in the intestinal epithelial cells[81]. Kraiczy *et al*[82] conducted a classification analysis of subcryptal mesenchymal cells in the small intestine and were the first to demonstrate that the BMP signaling gradient within the intestinal crypt architecture governs the self-organization of the Wnt-secreting stem cell niche.

Other regulators of ISCs

Signaling-mediated transcription regulation is key to ISC identity and fate, but growing evidence shows it works in tandem with immune cells, nutrition, and microbiome. ISC fate is regulated by cytokines, dietary factors, and microbial signals, which converge on core pathways that integrate niche signals.

Immune cells: IBD primarily results from dysregulation of the intestinal immune system. The intestinal epithelial barrier hosts a range of innate and adaptive immune cells, forming a key component of the gut mucosal immune system. Immune cells interact with ISCs both directly and through paracrine signaling. Pro-inflammatory cytokines such as interferon (IFN)-γ and IL-17 trigger Janus kinase/signal transducer and activator of the transcription 1 (STAT1) activation, leading to ISC apoptosis and impaired renewal. In contrast, regulatory T cells and IL-10 maintain ISCs viability *via* anti-apoptotic signaling[83]. Biton *et al*[51] used single-cell RNA sequencing to identify ISC subsets with high major histocompatibility complex II expression capable of interacting with CD4+ helper T cells and presenting antigens. Organoid studies showed

that pro-inflammatory T helper type 1 (Th1), Th2, and Th17 cytokines (IFN-γ, IL-13, IL-17) drive Lgr5+ ISC differentiation, while regulatory T cells and IL-10 promote their self-renewal[51]. *In vitro* co-culture of intestinal organoids with activated T cells results in ISC depletion and compromised organoid viability. Furthermore, in the absence of PCs, IFN-γ derived from CD4+ T cells directly trigger ISC apoptosis *via* the Janus kinase/STAT signaling pathway[84]. Recent studies have revealed a novel mechanism whereby integrin αEβ7 on intestinal T cells engages E-cadherin on ISCs, modulating downstream adhesion signaling to control epithelial proliferation and differentiation, thereby sustaining intestinal homeostasis[85]. Innate lymphoid cells (ILCs) produce various cytokines even under steady-state conditions, many of which directly influence epithelial cell function - most notably IL-22. ILC3s, as a key source of IL-22, support ISC maintenance and differentiation while providing protection against DNA damage[86]. Meanwhile, under IL-22-independent injury conditions, ILC3s drive intestinal repair by activating the transcriptional regulator YAP in transit-amplifying cells, leading to intestinal mucosal repair[87]. ILC2s and ILC1s also exert significant regulatory effects on ISCs. For example, ILC2-derived IL-13 activates IL-13Rα1 signaling in crypt ISCs, inducing Foxp1 expression to facilitate β-catenin nuclear translocation and sustain Lgr5+ ISC maintenance[88]. Using gut organoid-ILC1 cocultures, Jowett *et al*[89] showed that murine and human ILC1 secrete TGF-β1 to promote CD44v6+ crypt expansion and express matrix metalloprotease 9 to drive extracellular matrix (ECM) remodeling. In addition to T cells and ILCs, coculture of gut organoids with macrophages and dendritic cells highlights their critical roles in maintaining ISCs homeostasis. In summary, the intricate regulatory interactions between immune cells and ISCs play a pivotal role in maintaining the intestinal barrier and overall gut homeostasis.

Nutrition: ISCs play an underappreciated but emerging role as sensors of dietary nutrients, adjusting their fate decisions in response to nutritional status to help maintain gut homeostasis[90]. Distinct nutritional intervention - such as caloric restriction, fat,

and glucose - modulate ISC function and collectively regulate intestinal homeostasis. Caloric restriction amplifies the numbers and proliferation rates of Lgr5+ ISCs by suppressing mammalian target of rapamycin (mTORC) signaling in PCs, key components of the ISC niche, an effect that can be recapitulated by rapamycin[91,92]. Interesting, refeeding after fasting enhances ISC-associated tumor formation through the mTORC1-polyamine-protein synthesis axis[93]. Mattila *et al*[94] found that mTORC1 activation increases ISCs size in a region-specific manner, favoring the absorptive enteroblast lineage and inhibiting secretory EEC differentiation. Conversely, high-fat diet (60% fat) has been shown to enhance the proliferation and function of Lgr5+ ISCs and progenitor cells in the mammalian intestine[95]. Mechanistically, high-fat diet activates peroxisome proliferator-activated receptor delta, a ligand-dependent transcription factor that regulates metabolism and promotes self-renewal in ISCs and niche-associated progenitors. Short-term excess sucrose intake directly alters crypt cell metabolism and suppresses regenerative proliferation of ISCs and transit-amplifying cells[96].

Microbiome: The maintenance of intestinal homeostasis depends on the coordinated interaction between ISCs and the gut microbiota. Recent studies have revealed a complex interplay between ISCs and the gut microbiota, with microbial communities and their metabolites regulating ISCs function through multiple mechanisms. Gut microbiota species, such as *Lactobacillus* and *Bifidobacterium*, modulate ISC self-renewal and differentiation to support intestinal homeostasis and host defense against bacterial infection[97]. Salmonella infection in intestinal organoids leads to a marked reduction in Lgr5 and Bmi1 expression, key markers of ISCs. In contrast, *Limosilactobacillus reuteri* preserves Lgr5+ cell populations and promotes epithelial proliferation through RSPO activation[98]. Meanwhile, recent studies further emphasize that gut microbial metabolites - including short-chain fatty acids, lactate, succinate, indoles and their derivatives, and bile acids - can directly or indirectly influence ISC fate[99]. Furthermore, ISC self-renewal is regulated by gut microbiota and enteric serotonergic

neurons. Microbiota-derived valeric acid enhances tryptophan hydroxylase 2 expression by inhibiting nucleosome remodeling and deacetylase complex recruitment, leading to 5-hydroxytryptamine-mediated activation of prostaglandin E2 positive macrophages *via* 5-hydroxytryptamine (serotonin) receptor 2A/3A. Prostaglandin E2 then promotes Wnt/β-catenin signaling in ISCs through prostaglandin E2 receptor 1/4 receptors[100]. This study reveals complex interactions among the microbiota and their metabolites, enteric neurons, intestinal immune cells, and ISCs, uncovering a new layer of ISC regulation by niche cells and microbial signals.

ISC-BASED MECHANISMS IN IBD PATHOGENESIS

Inflammatory microenvironment in IBD

The inflammatory microenvironment in IBD plays a crucial role in the pathogenesis of both CD and UC, the two main types of IBD. This microenvironment is characterized by chronic inflammation, immune dysregulation, and interactions between immune cells, cytokines, and intestinal epithelial cells. First, In IBD, the persistent inflammation damages the intestinal epithelium, disrupts mucosal integrity, and drives disease progression[6]. The inflammatory environment directly harms intestinal epithelial cells, crucial for gut barrier formation. This damage results in ulcerations, crypt abscesses, and loss of the epithelial lining, intensifying the immune response and worsening gut inflammation. Secondly, IBD is marked by an aberrant immune response to normal gut flora, triggering immune activation against commensal bacteria. This dysregulation involves both innate and adaptive immune cells. Intestinal innate immunity is mediated by neutrophils, monocytes, macrophages, dendritic cells, ILCs, and natural killer cells, which are characterized by their ability to mount a rapid, nonspecific response as the first line of defense[101]. Compared to innate immune cells, adaptive immune cells exhibit high specificity and immunological memory, complementing each other to eliminate invading pathogens. In CD, Th1 and Th17 cells drive inflammation by secreting pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, IL-6, IL-17, IFN-γ, which recruit immune cells and worsen tissue damage[102]. Whereas Th2 cells

predominantly drive the inflammatory response in UC, secreting IL-4, IL-5, and IL-13, which contribute to tissue damage and mucus production[102]. In addition, dysbiosis or imbalance in the gut microbiota results in reduced microbial diversity and an overgrowth of pro-inflammatory bacteria[6]. In the IBD inflammatory microenvironment, immune cell-derived cytokines damage intestinal epithelial cells, compromising the epithelial barrier and allowing pathogens and luminal antigens to worsen inflammation. Furthermore, chronic inflammation in IBD activates fibroblasts and promotes the deposition of ECM proteins, such as collagen. This leads to intestinal fibrosis, which can cause strictures and impair the function of the affected region[103].

Epithelial barrier dysfunction

Epithelial barrier dysfunction and crypt destruction are hallmarks of IBD. The homeostatic repair and regeneration of the intestinal epithelium are controlled by ISCs located at the base of the crypts, which promote rapid turnover and generate various epithelial cell types. They differentiate into various cell types, including enterocytes (absorptive cells), goblet cells (mucus-secreting cells), and PCs (which support stem cell function and secrete antimicrobial peptides). The remaining stem cells following intestinal mucosal injury rapidly divide, replenish the stem cell population, proliferate, differentiate into mature intestinal epithelial cells, and repair the damaged mucosa.

In IBD, chronic inflammation impairs ISC function, resulting in compromised epithelial regeneration. Dysfunctional ISC activity results in decreased expression of epithelial tight junction proteins, which increases intestinal permeability and facilitates the translocation of harmful substances into the underlying tissue, thereby triggering an immune response[104]. In addition, Wnt and Notch signaling pathways are the most influential in regulating ISC function, especially in IBD. Studies have shown that in IBD patients, especially those with UC, increased Notch signaling and Wnt suppression lead to PC depletion, impairing the mucus barrier and ultimately compromising the intestinal barrier integrity[105,106]. Interesting, the intestinal epithelium’s response to inflammation involves not only stem and progenitor cells but also fully differentiated,

post-mitotic PCs. Inflammation-induced stem cell factor secretion activates c-Kit signaling, triggering a cascade that leads to glycogen synthase kinase 3β inhibition and Wnt activation in PCs[106]. Multiple studies have shown that inhibiting Notch signaling and promoting goblet cell differentiation can repair the mucosal barrier and alleviate colitis[107-109]. In recent years, growing evidence has highlighted the essential role of ISCs in maintaining epithelial barrier function. Such as DEAH-box helicase 9 deficiency in ISCs or PCs leads to R-loop accumulation, genomic instability, and cGAS-STING-mediated inflammation, compromising epithelial homeostasis[110]. Defects in the ISC niche lead to dysfunctional 3D organoid formation in mouse models of CD-like ileitis[111]. Conditional knockout of the sorting nexin 10 gene in intestinal epithelial cells or ISCs enhances intestinal mucosal repair in mouse colitis models, while also restoring ISC stemness[112].

Senescence and apoptosis of ISCs

The senescence and apoptosis of ISCs are pivotal mechanisms underlying the disruption of the intestinal epithelial barrier and disease progression in IBD. These processes compromise the gut's regenerative capacity and self-repair ability, while further perpetuating chronic inflammation. In senescence elevates the risk of IBD and colon cancer. The persistent lesions in the same intestinal region in CD may result from aged stem cells' inability to proliferate and generate new intestinal cells[113]. Mechanically, mTORC1 activation increases mitogen-activated protein kinases kinases 6 protein synthesis and enhances the activation of the p38 mitogen-activated protein kinases-p53 pathway, leading to a reduction in the number and activity of ISCs, as well as a decrease in villus size and density[114]. Furthermore, the gut microbiota can also influence the senescence of ISCs. Heat-inactivated *Bifidobacterium adolescentis* may improve colon senescence by enhancing the regeneration of ISCs both *in vivo* and *in vitro*, *via* the Wnt/β-catenin signaling pathway[115]. Meanwhile, exposure of colonic epithelial organoids to dextran sulfate sodium, oxazolone, or 2,4,6-trinitrobenzenesulfonic acid directly induced increased apoptosis and a depletion of

Lgr5+ cells[116]. Depletion of fucosyltransferase 2 in ISCs exacerbates endoplasmic reticulum stress and apoptosis, whereas fucosylated hypoxia up-regulated 1 enhances stem cell resistance to pro-apoptotic inositol-requiring enzyme 1 signaling[117]. Additionally, selective deletion of the methyltransferase 14 gene in the mouse colon led to apoptosis of colonic stem cells, resulting in mucosal barrier disruption and severe colitis[118].

Fibrosis

Intestinal fibrosis is a significant complication of IBD, occurring in both UC and CD, although it is more commonly seen in CD[119]. As fibrosis progresses, specific areas of the intestine become narrowed, leading to substantial damage to its structure and function, which significantly impairs patients' quality of life. Intestinal fibrosis is characterized by the excessive deposition of ECM components by activated cells derived from the mesenchyme in ISC niche. As an important regulatory component of the ISC niche, myofibroblasts play a key role in intestinal fibrosis. Following prolonged exposure to inflammatory stimuli, resident myofibroblasts undergo activation, a process governed by gradients of autocrine and paracrine soluble factors, including platelet-derived growth factor subunit A, platelet-derived growth factor subunit B, TGFβ1, insulin-like growth factor 1, and EGF, which are secreted by both immune and non-immune cells[120]. Nevertheless, myofibroblasts and mesenchymal cells are not the sole cell types implicated in intestinal fibrosis. Interestingly, non-mesenchymal cells, including endothelial and epithelial cells that have undergone endothelial-to-mesenchymal or epithelial-to-mesenchymal transitions, also play a role in ECM deposition during the fibrotic process[121,122]. In contrast to myofibroblasts, significant damage to and a decrease in the number of telocytes have been observed during fibrotic remodeling in IBD[123,124]. It is worth noting that LGR5+ ISC cells are markedly decreased in the fibrotic, stiffened gut[106,125]. However, He *et al*[126] noted that stiffening causes OLFM4 to extend into the villus regions in both the *in vitro* model and

the rigid human IBD colon. It provided evidence for the hypothesis that fibrosis-driven gut stiffening directly contributes to epithelial remodeling in IBD.

ISC: EMERGING KEY PLAYERS IN STEM CELL BASED IBD THERAPY

Mucosal healing in inflamed tissues could represent a promising target for improving clinical outcomes, reducing disease recurrence, and enhancing resection-free survival in patients with IBD[16,17]. Several existing treatments may exert protective or regenerative effects on the damaged epithelium, thereby facilitating mucosal healing. TNF-α inhibitors (biological agents) have induced mucosal healing in a subset of IBD patients, representing a substantial advancement in IBD therapy[127]. A significant number of patients do not respond to these biologic agents, highlighting the need for alternative therapies in IBD. As basic biological research and clinical trials advance, stem cell therapy is expected to expand the therapeutic options for IBD (Table 1).

Currently, clinical research on IBD primarily involves the use of HSCs or MSCs. HSCs can be extracted from peripheral blood, bone marrow, and umbilical cord blood, migrating directly to damaged tissues or differentiating into epithelial and immunomodulatory cells to restore normal mucosal structures[128]. In 2010, Burt *et al*[129] investigated the impact of autologous HSC transplantation (HSCT) on disease-free survival in patients with severe anti-TNFα refractory CD. Following treatment, all patients achieved a Crohn's Disease Activity Index of less than 150. The proportion of CD patients remaining relapse-free after transplantation was 91% at 1 year, 63% at 2 years, 57% at 3 years, 39% at 4 years, and 19% at 5 years. Although HSCT still holds clinical research value, studies on autologous HSCT in patients with refractory CD have indicated that while the procedure cannot address the high relapse rates associated with genetic susceptibility, it is also linked to a considerable frequency of serious adverse events[130-133]. As a result, the safety of HSCT in the treatment of IBD remains a critical concern. Compared to HSCT, autologous and allogeneic MSCs have a broader application in IBD patients. MSCs can be harvested from various human tissues, including bone marrow, adipose tissue, muscle connective tissue, periosteum, and

perichondrium[134]. MSCs regulate immune responses and control inflammation by secreting various soluble factors, such as cytokines, chemokines, and growth factors. These factors induce cell cycle arrest and apoptosis in lymphocytes, thereby promoting tissue regeneration[135]. Multiple clinical trials have demonstrated the safety and efficacy of both allogeneic and autologous bone marrow-derived MSCs in the treatment of CD, particularly perianal fistulas[136-140]. Furthermore, exosomes secreted by MSCs (MSCs-Exo) have also been explored for the treatment of IBD. In IBD mouse models induced by dextran sulfate sodium and 2,4,6-trinitrobenzenesulfonic acid, intraperitoneal administration of MSCs-Exo extracted from human umbilical cord MSC culture supernatant can restore mucosal barrier repair and maintain intestinal immune homeostasis in the mice[141]. Treatment with MSC-Exos reduced inflammatory responses, preserved intestinal barrier integrity, and promoted the polarization of M2b macrophages, without contributing to intestinal fibrosis[142].

In 2009, Sato *et al*[26] cultivated the first intestinal organoid using adult stem cells derived from mouse intestines, marking the beginning of the organoid research era. This study has demonstrated that Lgr5+ stem cells are both necessary and sufficient for the initiation and sustained growth of crypt-villus organoids[26]. Currently, there are two main approaches to establishing intestinal organoids: One involves isolating intestinal crypts directly from the donor for culture, while the other relies on *in vitro* differentiation of embryonic stem cells or induced pluripotent stem cells (iPSCs)[143]. Yui *et al*[144] showed that mouse colonic epithelial cells can be cultured as spherical aggregates in a 3D culture system, where they are embedded in a type I collagen gel, in the presence of specific cytokines. Furthermore, these spheres can be transplanted into sites of colonic epithelial damage in a UC model mouse. This study provides the first evidence that *ex vivo* cultured ISCs can be transplanted *in situ*, facilitating the regeneration of damaged mucosal tissue. Further studies revealed that organoids derived from fetal or adult small intestine can engraft onto damaged colonic epithelium, with differences in their adaptability to the surrounding environment due to cell plasticity[145]. Human intestinal organoids can also reconstruct the damaged

mucosa in immunodeficient mice[146]. Although the progenitor cells within organoids are sufficient to drive proliferation and differentiation, their limited plasticity and stemness ultimately constrain the diversity of cell types within the organoids. Recently, Yang *et al*[147] utilize a combination of small molecule pathway modulators to boost the stemness of organoid stem cells, thereby enhancing their differentiation capacity and increasing cellular diversity within human intestinal organoids. On the other hand, although stem cell-derived organoids are powerful models for epithelial function, they lack tissue-resident immune cells, which are crucial for capturing organ-level processes. Nikolche and his team successfully developed human intestinal immune organoids that incorporate autologous tissue-resident memory T cells, thereby modeling the interactions between intestinal epithelial cells and immune cells. Tissue-resident memory T cells actively infiltrate the organoids and integrate into the epithelial barrier, forming intestinal immune organoids that effectively recapitulate the immune microenvironment of the intestinal tissue *in vitro*[148].

Building on these previous studies, intestinal organoids can now be considered as one of the potential sources for repairing ulcers in patients with refractory IBD. We propose collecting ISCs from the patient's lesions *via* endoscopic biopsy, followed by *ex vivo* expansion using established organoid culture techniques. Once the required cell quantity is reached, they can be transplanted to the target site using an endoscopic delivery approach (Figure 3). However, many challenges remain in establishing organoid transplantation, including the development of an endoscopic cell delivery system, the quality of cultured ISCs, the identification of optimal indices to evaluate the clinical efficacy, and the tumorigenicity of donor organoids, among others[149]. Therefore, intestinal organoid transplantation for the treatment of IBD requires extensive clinical trials in the future for evaluation.

FUTURE PERSPECTIVES

Meanwhile, advances in gene editing, stem cell engineering, and artificial intelligence (AI) are reshaping ISC research, creating new avenues for disease modeling and

therapeutic innovation through their interdisciplinary integration. For instance, the combination of human iPSC and CRISPR-Cas9 technology offers a versatile and powerful platform for gene editing, enabling the investigation of disease mechanisms and the discovery of therapeutic targets. Using a lentiviral vector expressing single-guide RNA and CRISPR-Cas9, Sens *et al*[150] generated iPSC lines with targeted knockouts of IL-10RA, IL-10RB, and the downstream signaling molecules STAT1 and STAT3. In parallel, AI is emerging as a powerful tool in the diagnosis, monitoring, and management of IBD. Deep learning algorithms applied to endoscopic images and histopathological slides have demonstrated excellent performance in lesion detection, disease classification, and treatment response prediction[151,152]. Therefore, we propose that integrating AI with ISC-based therapies can further enhance clinical outcomes by enabling dynamic monitoring of epithelial regeneration and early relapse detection. Additionally, AI has the potential to accelerate research by analyzing complex data from ISC-derived organoids, revealing novel phenotypes and identifying therapeutic targets with greater speed and accuracy than traditional methods.

CONCLUSION

Initial studies suggested CBCs as progenitors of the intestinal epithelium, and subsequent lineage-tracing confirmed Lgr5+ CBCs as ISCs capable of self-renewal and multilineage differentiation. Additional markers, including CD133, Olfm4, achaete-scute homolog 2, and SRY-related high-mobility group box 9, have refined CBC identification. Quiescent +4 cells, located above PCs, serve as reserve ISCs with high radiation resistance, replenishing CBCs when needed. Markers like Bmi1, Hopx, telomerase reverse transcriptase, and leucine-rich repeats and immunoglobulin-like domains containing protein 1 are associated with +4 cells. Recent studies challenge the exclusive role of Lgr5+ CBCs in regeneration, highlighting Fgfbp1+ cells in the upper crypt as multipotent progenitors that contribute to ISC renewal, thus broadening the concept of ISC plasticity and regenerative capacity. ISCs are regulated by a complex niche comprising stromal and epithelial cells, such as PCs, fibroblasts, and immune

cells, which govern ISC proliferation, differentiation, and survival. Key signaling pathways, including Wnt/β-catenin, EGF, Notch, and BMP, coordinate ISC function and fate. Wnt/β-catenin signaling drives ISC proliferation, while EGF and Notch pathways regulate differentiation. BMP signaling inhibits proliferation and promotes differentiation. Additionally, immune cells, nutrition, and microbiota modulate ISC activity. Impaired function of ISCs hampers epithelial repair, undermining the gut barrier and enhancing permeability. Dysregulation of Wnt and Notch signaling pathways exacerbates ISC dysfunction. Additionally, ISC senescence and apoptosis further diminish regenerative potential. Intestinal fibrosis arises from the activation of myofibroblasts and excessive ECM deposition, resulting in intestinal stiffness and further impairment of ISC activity, which disrupts epithelial homeostasis.

The treatment of IBD has predominantly focused on immunosuppression. Many of these medications, especially steroids and immunomodulators, exert widespread immunosuppressive effects, which can result in an increased risk of infections and neoplastic complications. Stem cell-based therapies are emerging as promising treatments for IBD. HSCs and MSCs are the primary candidates, with HSCT showing potential but also high relapse rates and safety concerns. MSCs, through cytokine secretion and exosome-based mechanisms, promote tissue regeneration and modulate inflammation, demonstrating efficacy in IBD treatment. Additionally, intestinal organoids, derived from Lgr5+ stem cells, offer significant potential for mucosal repair. Recent innovations in organoid culture, including enhanced stemness and immune cell integration, bolster their therapeutic potential for refractory IBD. Despite these advances, challenges remain in optimizing cell delivery systems, evaluating clinical efficacy, and addressing tumorigenicity. Extensive clinical trials are necessary to fully assess the viability of organoid-based therapies for IBD. We suppose that ISC transplantation into the inflamed mucosa through endoscopic delivery approach will provide a new therapeutic approach to reconstruct the epithelial barrier in IBD.

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