THERAPEUTIC ADVANCES in
Hematology

Review

Research advances on short-chain fatty acids in gastrointestinal acute graft-*versus*-host disease

Ther Adv Hematol

2024, Vol. 15: 1–13

DOI: 10.1177/20406207241237602

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***Abstract*:** Gastrointestinal acute graft-*versus*-host disease (GI-aGVHD) is a severe early complication following allogeneic hematopoietic stem cell transplantation (allo-HSCT). It has been shown that the intestinal microbiota plays a critical role in this process. As metabolites of the intestinal microbiota, short-chain fatty acids (SCFAs) are vital for maintaining the host-microbiota symbiotic equilibrium. This article provides an overview of the protective effect of SCFAs in the gastrointestinal tract, emphasizes their association with GI-aGVHD, and explores relevant research progress in prevention and treatment research.

Plain language summary

Research advances on short-chain fatty acids in gastrointestinal acute graft-versus-host disease

Gastrointestinal acute graft-versus-host disease (GI-aGVHD) is a severe early complication following allogeneic hematopoietic stem cell transplantation (allo-HSCT). It has been shown that the intestinal microbiota plays a critical role in this process. As metabolites of the intestinal microbiota, short-chain fatty acids (SCFAs) are vital for maintaining the host-microbiota symbiotic equilibrium. This article provides an overview of the protective effect of SCFAs in the gastrointestinal tract, emphasizes their association with GI-aGVHD and explores relevant research progress in prevention and treatment research.

Keywords: acute graft-*versus*-host disease, allogeneic hematopoietic stem-cell transplantation, short-chain fatty acids

Received: 6 November 2023; revised manuscript accepted: 19 February 2024.

Introduction

Allogeneic hematopoietic stem cell transplantation (allo-HSCT) is currently recognized as a crucial therapeutic approach for blood tumors, bone marrow failure, immunodeficiency diseases, and even considered the sole method of curing certain ailments.[1] Graft-*versus*-host disease (GVHD) was defined as a syndrome in which the immune-active cells from the donor recognize and attack the immunocompromised host tissues of an allogeneic recipient.[2,3] However, acute GVHD (aGVHD) still affects 35–55% of human leukocyte antigen (HLA)-matched sibling transplant recipients, with even higher occurrence rate in unrelated donor transplants.[4] This significantly impedes the success rate of allo-HSCT and severely impacting patients' quality of life and prognosis. The gastrointestinal tract is believed to be the second most affected target organ in aGVHD and is implicated in various complications.[5,6]

The majority of symbiotic bacteria in the human body reside in the colon,[7] predominantly comprising the phyla Bacteroidetes, Firmicutes, Proteobacteria, and Actinobacteria.[8] These

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intestinal symbiotic bacteria ferment indigestible dietary fibers to produce short-chain fatty acids (SCFAs), primarily acetate, propionate, and butyrate, with acetate and propionate being mainly produced by Bacteroidetes and butyrate by Firmicutes.[9] Most SCFAs are absorbed, metabolized, and contribute to maintaining intestinal homeostasis.[9,10] This review outlines the protective role of SCFAs in the gastrointestinal tract, discusses the latest advances in understanding their association with gastrointestinal acute graft-*versus*-host disease (GI-aGVHD), and explores relevant intervention measures.

Protective mechanisms of SCFAs in the intestinal tract

SCFAs facilitate proliferation and restoration of intestinal epithelial cells

The intestinal epithelium consists of a continuously regenerating layer of intestinal epithelial cells (IECs), serving as the primary defense against intestinal infections. The proliferation, differentiation, and migration of the epithelial layer rely on numerous growth signals and energy resources. SCFAs have been demonstrated to play a crucial role in these processes. Over 90% of SCFAs are efficiently absorbed by IECs from the intestinal lumen, participating in energy metabolism.[11] Among them, butyrate salts serve as the principal source of energy, with their oxidative metabolism accounting for approximately 73–75% of oxygen consumption in human colonic cells.[12] In instances of energy deficiency, IECs undergo autophagy although this can be reversed through supplementation of symbiotic bacteria or direct administration of SCFAs, demonstrating that gut symbiotic bacteria promote IECs proliferation activity and facilitate the restoration of the intestinal mucosa through SCFAs-mediated mechanisms.[13,14]

In addition, the human genome encodes six potential G-protein coupled receptors (GPCRs) that are sensitive to SCFAs. These include GPR41 (FFAR3), GPR42, GPR43 (FFAR2), GPR109a (HCAR2), GPR164 (OR51E1), and OR51E2. Among them, GPR41 and GPR43 specifically recognize acetic acid, butyric acid, and propionic acid, while GPR109a is activated only by butyric acid.[12] The research team employed videomicroscopy and single-cell tracking to unveil that propionic acid inhibits histone deacetylases (HDACs) of Class I. Furthermore, this inhibition relies on GPR43, signal transducer, and activator of transcription 3 (STAT3), which enhance cell migration and polarization, ultimately promoting intestinal epithelial migration and facilitating intestinal epithelial repair.[15]

SCFAs preserve intestinal mucosal barrier integrity and regulate immune homeostasis

Hypoxia-inducible factor 1α (HIF-1α) is a critical regulatory factor in mammalian oxygen homeostasis. It can stabilize and upregulate the transcription of Claudin1, a tight junction membrane protein that enhances epithelial barrier integrity and reduces intestinal inflammation.[16] Kelly *et al.*[17] discovered that butyrate salts within SCFAs can induce oxidative respiration in colonic cells, leading to physiological hypoxia and subsequent stabilization of HIF-1α, thereby reducing intestinal barrier permeability. In subsequent studies, Fachi *et al.*[18] found that butyrate salts upregulate tight junction proteins in an HIF-1α-dependent manner, improving barrier integrity and suppressing microbial translocation in mice, resulting in reduced inflammation.

Another important mechanism related to mucosal barrier is the production of antimicrobial peptides (AMPs) by IECs and Paneth cells, such as defensins and regenerating islet-derived proteins (Reg) family. Various microbial-associated molecular patterns, including LPS, peptidoglycans, flagella, bacterial DNA/RNA, fungal cell wall components, can induce the expression of AMPs and other mucosal adaptive immune components (such as IgA).[19] Among the RegIII lectin family, RegIIIγ is the most widely expressed AMP in the small intestine, IECs can generate interleukin-33 (IL-33) upon injury, thereby promoting the production of RegIIIγ.[20] Butyrate, through the activation of the GPR43 pathway, effectively induces the expression of RegIIIγ and β-defensin in IECs both *in vitro* and *in vivo* mouse models, thereby regulating mucosal barrier.[21] Additionally, it is noteworthy that the intriguing combination of RegIIIα (a member of the Reg family) and ST2 (the soluble receptor for IL-33) has been incorporated into the MAGIC algorithm probability, which has demonstrated its efficacy in accurately predicting the estimated probability of nonrelapse

mortality in aGVHD patients at the 6-month mark.[22]

SCFAs also participate in the regulation of the immune system, maintaining a balance between intestinal pro-inflammatory and anti-inflammatory effects. Studies have revealed that SCFAs not only rely on HDAC inhibitory activity but also selectively induce Treg differentiation in a GPR43-dependent manner, thereby modulating intestinal inflammatory responses and alleviating the development of aGVHD.[23–26] Interestingly, butyrate salts promote the generation of new Treg cells without promoting their accumulation in the colon, while acetate salts exhibit the opposite activity, and propionate salts possess both properties simultaneously.[23]

Moreover, SCFAs promote the expression of IL-10 in Th1 cells, dendritic cells, and macrophages through their interaction with GPR43 or GPR109a, effectively alleviating intestinal inflammation.[27,28] SCFAs also stimulate K+ efflux and hyperpolarization through the aforementioned pathways, activating NLRP3 inflammasome and facilitating the production of IL-18, a cytokine that promotes intestinal epithelial barrier function.[29,30]

Similar to GPR43 and GPR109a, the activation of other SCFA receptors such as GPR41 induces intracellular Ca^{2+} mobilization, subsequently activating the inflammasome to exert its inflammatory effects.[29] The absence of GPR43 in mice exacerbates inflammation in models such as dextran sulfate sodium (DSS)-induced colitis.[31] Similarly, mice lacking NLRP3 inflammasome or IL-18 develop aggravated colitis in the DSS model.[30] Under high-fiber feeding, a substantial production of SCFAs is observed, which can activate GPR109a and GPR43, subsequently activating NLRP3 and leading to the release of IL-18. This process promotes intestinal epithelial repair and protects against the development of colitis. Similarly to GPR43 and GPR109a, activation of other SCFA receptors such as GPR41 induces intracellular Ca^{2+} mobilization, resulting in inflammasome activation.[29]

In summary, these findings collectively underscore the crucial role of IECs in establishing a physical, chemical, and immune barrier between the intestinal environment and the host's symbiotic microbiota through SCFAs. This barrier plays a vital role in maintaining homeostasis, controlling intestinal inflammation, and even influencing the occurrence and development of GI-aGVHD.

SCFAs sustain gut microbiota colonization resistance and participate in regulating gut microbiota homeostasis

The gut microbiota (GM) also serves as a reservoir for multidrug-resistant organisms (MDROs).[32] A healthy microbiota can prevent the expansion of pathogens through direct bacterial–bacterial interactions or by activating host immune defenses, a phenomenon known as colonization resistance.[33]

SCFAs play a pivotal role in this process. Previous mouse studies have shown that SCFAs can mediate the peroxisome proliferator-activated receptor-gamma pathway to regulate energy metabolism in IECs, reducing the utilization of oxygen and nitrate by Enterobacteriaceae bacteria, thereby indirectly counteracting dysbiosis and expansion.[34] Not only can they modulate the sensitivity of Enterobacteriaceae colonization in antibiotic-disrupted ecosystems, but they also exhibit such effects in undisturbed microbiota communities.[35] In contrast, long-term observations have revealed that antibiotic treatment during *Salmonella gastroenteritis* recovery period sometimes leads to bacterial and symptomatic relapse. This has been attributed to the depletion of butyrate-producing *Clostridium* species by both *Salmonella* virulence factors and antibiotics, resulting in increased aerobic expansion of *Salmonella* facilitated by enhanced oxygenation of IECs.[36] Recent studies have demonstrated that in the acidic environment of the gut, SCFAs directly mediate intracellular acidification, effectively inhibiting the abnormal expansion of antibiotic-resistant pathogens such as *Klebsiella pneumoniae* and *Escherichia coli*. Furthermore, in patients undergoing allo-HSCT, a decrease in SCFAs levels has been observed, correlating with subsequent expansion of *E. coli* in the intestine and leading to the development of bloodstream infections.[37] Recently, it has been discovered that *Lactobacillus* creates an antagonistic environment for the growth of MDROs by increasing the level of butyrate produced by *Clostridium*, which is considered a key factor in limiting the colonization of MDROs in the gut.[38]

In conclusion, under physiological conditions, SCFAs can reduce pathogen colonization through indirect or direct mechanisms, thereby establishing and maintaining a stable gut environment. However, when various factors lead to a depletion or exhaustion of SCFA-producing microbial populations in the host's gut, it favors the abnormal expansion of aerobic bacteria and increases the risk of infection.

The relationship between GI-aGVHD and SCFAs

Changes in the intestinal microbiota during hematopoietic stem cell transplantation

Significant alterations in intestinal microbiota diversity occur during allo-HSCT.[39,40] A recent large-scale multicenter observational study, utilizing 16S rRNA gene sequencing to analyze the microbial composition of 8767 stool samples from 1362 patients undergoing allo-HSCT, reported similar findings. The disruption of GM during allo-HSCT exhibits similarities across transplant centers and geographical locations. This disruption is characterized by a loss of GM diversity and dominance of a single taxonomic group. Lower GM diversity is associated with higher transplant-related mortality and increased risk of GVHD-related mortality. Furthermore, pre-transplant samples already exhibit signs of GM dysbiosis, as lower pre-transplant GM diversity correlates with poorer survival rates. On the day of hematopoietic stem cell infusion, the GM structure of many patients already significantly deviates from that of healthy volunteers, demonstrating microbial disruption.[41] Recent scholars have suggested that a more stable arrangement of GM during allo-HSCT is associated with a shorter duration of febrile neutropenia.[42] Patients with lower GM diversity at the time of neutrophil engraftment exhibit higher mortality rates, suggesting potential clinical predictive indicators for allo-HCT mortality.[40,41]

Specifically, based on 16S rRNA sequencing technology, several centers have successively reported similar findings: samples with lower GM diversity are characterized by a significant increase or dominant presence of relative abundance in genera such as *Enterococcus*, *Streptococcus*, *Escherichia*, *Klebsiella*, *Lactobacillus*, and *Staphylococcus*.[39–41,43] At the species level, Ilett *et al.*[44] utilized metagenomic sequencing techniques to reveal an enrichment phenomenon of *Enterococcus faecium*, *Lactobacillus delbrueckii*, *Staphylococcus epidermidis*, and *Streptococcus thermophilus*.

In patients with aGVHD, more pronounced alterations in the microbial community structure of the intestinal tract are observed. At the family level, there is a significant decrease in Borreliaceae and Ruminococcaceae within the phylum Spirochaetes. At the genus level, reductions are observed in *Lachnoclostridium*, *Blautia*, *Sellimonas*, and *Anaerostipes* of the family Borreliaceae, Faecalibacterium UBA181 and Flavonifractor of the family Ruminococcaceae, Erysipelatoclostridium of the family Erysipelotrichaceae, and Lactococcus of the family Streptococaceae. Additionally, depletion of *Akkermansia muciniphila* (*A. muciniphila*) of the phylum Verrucomicrobia is also observed.[43,44] Among them, the higher abundance of *Blautia* and *A. muciniphila* is considered a protective factor against GVHD, while their reduction has been demonstrated to be significantly associated with the occurrence of aGVHD.[44]

The relationship between SCFA levels and aGVHD

The structural changes in GM during the aforementioned HSCT are accompanied by significant alterations in its metabolic byproducts, SCFAs. During GI-aGVHD, there is a sharp decline in intestinal SCFA production in both adult and pediatric patients.[43,45] The levels of propionate and acetate are correlated with the severity of GI-aGVHD, while butyrate exhibits a significant decrease throughout all stages of aGVHD, suggesting its potential as a diagnostic biomarker for GI-aGVHD.[43] It is noteworthy that an increased abundance of certain butyrate-producing bacteria, particularly *Clostridium difficile*, is beneficial for the prognosis of aGVHD.[46]

SCFAs have been demonstrated to play a protective role in GI-aGVHD. Mathewson *et al.* discovered a significant reduction in the expression of butyrate monocarboxylate transporter (SLC5A8) and butyrate receptor (GPR43) in mouse intestinal tissues after HSCT. This reduction was accompanied by decreased levels of butyrate and histone acetylation in IECs. However, supplementation with exogenous butyrate or colonization with butyrate-producing strains of *C. difficile*

increased the uptake of butyrate and histone acetylation in IECs. This led to improved IEC integrity, alleviating aGVHD and ultimately enhancing survival rates.[47] Subsequent investigations have further revealed that butyrate and propionate exert their GVHD-alleviating effects by binding to GPR43 on IECs, thereby activating ERK phosphorylation and NLRP3 inflammasome, leading to increased production of IL-18.[48]

Consequently, the alterations in GM and their metabolic byproducts, SCFAs, during HSCT have been linked to the prognosis of GI-aGVHD. The loss of GM diversity during allo-HSCT coincides with decreased levels of intestinal SCFAs. This, in turn, affects the intestinal mucosal defense mechanisms, leading to a redistribution of GM structure, promoting intestinal inflammation, and facilitating the colonization and translocation of MDROs into the bloodstream. This occurrence increases the risk of severe infections and complications, ultimately exacerbating the development of aGVHD and contributing to unfavorable outcomes.[49] However, when GI-aGVHD has already occurred and caused intestinal mucosal damage, there are differing views among researchers. Some argue that butyrate may hinder the recovery of the intestinal mucosa, thereby increasing the risk of refractory and chronic GVHD.[50] Recent studies have indicated that transplantation with GPR109a-deficient (a specific GPCR that binds butyrate) T cells can enhance the abundance of SCFA-producing bacteria, reduce IEC damage, and decrease the risk of GVHD occurrence by 50%.[51] Further research is needed to explore the specific mechanisms underlying this process.

The factors influencing the levels of SCFAs

The impact of antibiotic usage during HSCT on SCFAs levels

One clear cause of dysbiosis in the GM is the usage of antibiotics, which can be traced back to as early as the 1970s when extensive studies in mouse models demonstrated the impact of antibiotics on the microbial community.[32] During allo-HSCT, the reduction of neutrophils due to myeloablative conditioning regimens and mucosal damage often leads to neutropenic infections.[52] Most patients receive prophylactic and therapeutic antibiotics during the neutropenic phase, which typically occurs in the first week after allo-HSCT.[53] Recent research findings warrant attention: antibiotic exposure is identified as the primary driving factor for microbial community changes during HSCT, rather than alloreactivity, intensity of conditioning, or immunosuppression.[54]

Extensive research has revealed that antibiotics with activity against gut commensal bacteria involved in SCFA production can increase the risk of GVHD. For instance, compared to aztreonam or cefepime, piperacillin–tazobactam or imipenem–cilastatin exacerbates dysbiosis and is significantly associated with higher GVHD-related mortality.[55] Exposure to clindamycin is believed to be associated with depletion of anti-inflammatory Clostridia in the gut of pediatric patients and worsened GVHD, a conclusion supported by subsequent mouse model validations where oral supplementation of Clostridia probiotics alleviated GVHD.[56] Additionally, patients who receive early antibiotic therapy experience worse clinical outcomes compared to those who receive broad-spectrum antibiotics later or not at all.[52] Through measurement of SCFAs concentrations during HSCT, the research team discovered that patients with higher exposure to antibiotics targeting anaerobic bacteria exhibited significant reductions in butyrate and propionate levels in the intestinal lumen, which correlated with depletion of Firmicutes and other anaerobic bacteria (particularly *Akkermansia*) and increased GVHD incidence.[45] At the molecular biology level, Ghimire *et al.*[57] found that broad-spectrum antibiotic treatment during HSCT is an independent factor leading to diminished expression of SCFA sensing receptors (GPR109A, GPR43, and FOXP3) which are implicated in mitigating GVHD.

Therefore, the type and timing of antibiotic administration have a critical impact on the composition of the GM, concentration of SCFAs, and transplantation outcomes. This consideration should not be limited to the period of HSCT alone but should extend to the pre-transplant phase as well. It is essential to carefully select and administer antibiotics based on individual clinical circumstances for both adult and pediatric patients. This approach aims to maintain the stability of the GM structure and SCFA concentrations to minimize the risk of aGVHD and adverse outcomes.

The impact of nutritional changes on SCFAs levels during hematopoietic stem cell transplantation

The majority of allo-HSCT patients have relatively healthy nutritional status before pre-treatment,[58–60] but it rapidly deteriorates after therapy.[61,62] This is due to treatment-related side effects such as nausea, vomiting, and diarrhea, as well as transplant-related complications including infections, GVHD, and sinusoidal obstructive syndrome of the liver.[63–65] Over time, increased catabolic metabolism, inadequate oral intake, poor gastrointestinal absorption, and compromised nutritional status contribute to varying degrees of malnutrition, further increasing the risk of severe GVHD in patients.[66,67] Evidence indicates that changes in dietary patterns not only play a significant role in altering the relative and absolute abundance of gut bacteria, but also impact their growth kinetics.[68] Therefore, implementing nutritional support during hematopoietic stem cell transplantation (HSCT) and aiming to maintain the balance of GM has become a focal point of research interest.

Parenteral nutrition (PN) is currently considered the primary method of nutritional support in most transplantation centers.[69] However, it is associated with various adverse reactions such as infections, intestinal mucosal atrophy, and alterations in GM composition. Prolonged duration of PN has been shown to be related to the loss of *Blautia* genus, even in patients who avoid the use of antibiotics targeting anaerobic bacteria.[46]

On the contrary, early enteral nutrition (EN) is believed to improve the prognosis of allo-HSCT patients, with a more significant impact on the occurrence of gastrointestinal GVHD compared to cutaneous or hepatic GVHD.[70] Even in patients receiving combined EN and PN, the incidence of GI-aGVHD, hypoalbuminemia, and electrolyte imbalance remains higher than in those receiving EN alone.[71] In a longitudinal analysis conducted by D'Amico *et al.*,[72] it was discovered that SCFAs significantly increased in post-transplantation stool samples only in the EN group, indicating that adequate provision of EN during HSCT has the potential to facilitate the restoration of GM structure and contribute to mitigating the risk of aGVHD. However, EN's primary limitation lies in the challenge of implementing tube feeding in patients with severe mucositis or gastrointestinal injury.[73] Therefore, as per recent international guidelines, EN support should be employed for patients with functional gastrointestinal capacity but inadequate oral intake to meet their nutritional requirements. In cases of intractable vomiting, intestinal obstruction, severe malabsorption, and similar circumstances, PN may be selected. PN usage should be discontinued after stem cell engraftment when EN or sufficient oral intake can be maintained.[74]

The application of interventions targeting SCFAs during HSCT

In general, current clinical interventions aim to manipulate SCFAs levels from three perspectives: (1) Indirect modulation through dietary intake of substances that act as substrates for GM, such as prebiotics. (2) Direct modulation by introducing or eliminating specific bacterial strains, such as consuming probiotics or utilizing bactericidal agents targeting sensitive species. (3) Reshaping the microbial structure through fecal microbiota transplantation (FMT).

Prebiotics

Prebiotics are defined as 'a substrate that is selectively utilized by host microorganisms conferring a health benefit'. Examples include resistant starch, oligofructose, and oligogalactose.[75] The GM can utilize prebiotics to ferment and produce SCFAs. Recent studies have reported that supplementation with oligogalactose promotes butyrate production, leading to increased survival rates and alleviation of GVHD symptoms in hematopoietic stem cell-transplanted mice following antibiotic treatment.[76] Yoshifuji *et al.* conducted a study in which they supplemented HSCT patients with a mixture of resistant starch and a prebiotic blend, containing glutamine, fiber, and oligosaccharide (GFO). They observed that the supplementation helped maintain GM diversity, increased the relative abundance of butyrate-producing bacterial species, and preserved fecal butyrate levels. Furthermore, it resulted in a shortened duration of moderate to severe oral mucositis (OM) and diarrhea, as well as a reduced incidence of grade II–IV aGVHD.[77] Hence, the intake of prebiotics may be considered as one effective strategy for preventing aGVHD in HSCT patients. However, there is currently insufficient evidence to support the

optimal dosage and timing of prebiotic intake in allo-HSCT patients. The selection and safety of prebiotics still require further validation and evaluation.

Probiotics

Probiotics are defined as 'live microorganisms that, when administered in sufficient quantities, confer a health benefit on the host'.[78] Based on the aforementioned, it is theoretically feasible to improve clinical outcomes by directly administering live microorganisms to regulate the balance of the gut ecosystem. In fact, studies have been conducted to explore the safety of probiotic preparations in allo-HSCT patients, yielding mixed results. On one hand, mouse studies have indicated that *Lactobacillus rhamnosus* GG (LGG) is beneficial in alleviating aGVHD and reducing post-transplant mortality.[79] A study analyzed blood cultures from 3796 recipients of HSCT and observed a low incidence rate (0.5%) of bloodstream infections related to common probiotic bacteria.[80] Ladas *et al.*[81] prospectively investigated the safety of orally administering *Lactobacillus plantarum* (LBP) to children and adolescents undergoing neutropenia during HSCT, and no occurrences of LBP bacteremia or adverse events related to LBP were observed. Recently, viable Bifidobacterium tablets have been demonstrated to effectively reduce the incidence and duration of OM at grades I–II, without affecting engraftment rate.[82] However, on the other hand, another research group found that supplementation with LGG did not provide protective effects against GVHD in HSCT patients.[83] Furthermore, Koyama *et al.*[84] reported a case of septic shock in an autologous stem cell transplant recipient caused by the consumption of yogurt containing LGG during a bout of diarrhea. Therefore, the selection and timing of probiotic preparations for immunocompromised HSCT patients still require cautious and targeted consideration.

Fecal microbiota transplantation

In recent years, FMT has emerged as a novel approach for modulating the composition of the GM, and numerous studies have investigated its application in HSCT. In 2018, Taur *et al.*[85] conducted a clinical randomized controlled trial that demonstrated the restoration of pre-transplant baseline levels of GM diversity and composition in HSCT patients through the use of autologous FMT. Subsequently, van Lier's team administered unrelated healthy donor fecal suspension to 15 patients with steroid-refractory GI GVHD as a treatment using FMT. Within 1 month after FMT, 10 individuals exhibited increased GM diversity and elevated levels of butyrate-producing bacteria. Among them, six patients were able to gradually reduce their immunosuppressive medication.[86] Therefore, despite the immunocompromised state of HSCT patients, FMT has the potential to restore a symbiotic microbial community, increase GM diversity to relatively safe levels, and effectively counteract the progression of GI GVHD. However, adverse reactions related to FMT, including diarrhea, bloating, abdominal pain, vomiting, and infections, cannot be disregarded.[87] Furthermore, reports have emerged on FMT-related bacteremia in different patients, demonstrating a connection to the same stool donor, with one HSCT patient unfortunately experiencing fatal consequences.[88] Rigorous donor screening is a necessary measure. The feasibility, safety, and long-term effects of FMT in HSCT patients still require exploration through large-scale clinical studies.

Conclusion

In summary, a wealth of evidence indicates that during HSCT, various factors such as chemotherapy drugs, antibiotics, and malnutrition lead to dynamic changes in the GM composition. The loss of GM diversity is accompanied by a decrease in the levels of SCFAs, its metabolic byproducts. This results in decreased stability of the intestinal mucosal barrier, gradual dominance of certain bacterial species, exacerbation of intestinal inflammation, and translocation of bacteria into the bloodstream, ultimately affecting the occurrence and development of GI-aGVHD and even bacteremia. However, it is worth noting that the occurrence of the aforementioned adverse outcomes may be the result of multiple factors acting collectively, including the infection itself and other related conditions, rather than solely the loss of GM diversity itself.

Predicting, evaluating, and intervening in aGVHD through the microbiome–metabolome axis has become a feasible approach. Monitoring the levels of SCFAs in the gut of HSCT patients may be one method. Commonly utilized samples for the detection of SCFAs include fecal samples,

intestinal tissues, and blood. Due to the non-invasive nature of the collection process and its relative convenience, the application of fecal samples is more widespread.[89] Methods for quantifying the levels of SCFAs are diverse, including gas chromatography (GC), mass spectrometry (MS), high-performance liquid chromatography (HPLC), ultraviolet detection, electrochemical detection, and capillary electrophoresis. Among them, GC/MS is commonly used for the determination of SCFAs in biological samples due to its higher sensitivity.[90] Additionally, reports have indicated the potential use of positron emission tomography (PET) tracers (such as ^{18}F-FDG and ^{18}F-FPIA) for imaging the metabolic activity and distribution of SCFAs in the human body.[91,92] Nevertheless, incorporating SCFA levels as a predictive indicator may pose certain challenges in clinical practice. As mentioned earlier, the basal levels of SCFAs are influenced by various factors, leading to individual variations. If the SCFAs level is considered as a predictive indicator, the results of a single test may not accurately reflect the occurrence and progression of GI GVHD. In fact, conducting multiple tests would also entail additional costs in terms of time and finances for the patients. Furthermore, there exists a biological gradient of SCFAs from the intestinal lumen to the periphery.[89] In certain specific circumstances, the results of intestinal biopsy may provide a more precise assessment. Nevertheless, as an invasive procedure, the acceptance of intestinal biopsy remains closely tied to patient receptiveness. Hence, further research is needed to optimize SCFA detection methods that offer high accuracy, relatively simple sampling and cost-effectiveness, which will bring broad benefits.

Strategies for modulating the GM have been preliminarily explored during HSCT. In the future, it may be worthwhile to conduct *in vitro* and *in vivo* experiments using SCFA formulations (such as butyrate preparations) for the prevention and treatment of GI GVHD, providing new insights for GI GVHD management. Additionally, emerging biologic therapies, including mesenchymal stem cells (MSCs), Janus kinase inhibitors (JAK inhibitors), Bruton's tyrosine kinase inhibitors (BTK inhibitors), and Rho-associated protein kinase inhibitors (ROCK inhibitors), have shown certain clinical efficacy in alleviating steroid-refractory GVHD patients.[93–95] Recent studies have demonstrated that MSCs can enhance SCFA production by upregulating the abundance of SCFA-producing bacteria, thereby regulating T cell immune homeostasis and improving colonic inflammation.[96] However, our understanding of whether biologic therapies can induce changes in the gut microbiome and their functional implications in GVHD patients remains limited, warranting further investigation. In conclusion, further large-scale multicenter studies are still needed to assess the changes in the microbiota and its metabolites during HSCT, as well as the safety, efficacy, standardized procedures, and long-term adverse reactions of various intervention measures, in order to improve the prognosis of HSCT patients.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Author contributions
Xinping Song: Investigation; Supervision; Writing – original draft; Writing – review & editing.

Jing Lao: Investigation; Supervision.

Lulu Wang: Writing – review & editing.

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Acknowledgements
The authors thank all members of our laboratory for their valuable discussions.

Funding
The authors disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This research was funded by The Medical Science and Technology Foundation of Guangdong Province (A2023335), Sanming Project of Medicine in Shenzhen (SZSM201512033), Guangdong Medical Science and Technology Research Project (A2020101), Shenzhen Fund for Guangdong Provincial High-level Clinical Key Specialties (SZGSP012), Shenzhen Key Medical Discipline Construction Fund

(SZXK034), Shenzhen Healthcare Research Project (SZLY2018015).

Competing interests

The authors declare that there is no conflict of interest.

Availability of data and materials

Not applicable.

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