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Review article: fungal microbiota and digestive diseases

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SUMMARY

Background
The role of the fungal microbiota in digestive diseases is poorly defined, but is becoming better understood due to advances in metagenomics.

Aim
To review the gastrointestinal fungal microbiota and its relationship with digestive diseases.

Methods
Search of the literature using PubMed and MEDLINE databases. Subject headings including 'fungal-bacterial interactions', 'mycotoxins', 'immunity to fungi', 'fungal infection', 'fungal microbiota', 'mycobiome' and 'digestive diseases' were used.

Results
The fungal microbiota is an integral part of the gastrointestinal microecosystem with up to 10^6 microorganisms per gram of faeces. Next-generation sequencing of the fungal 18S rRNA gene has allowed better characterisation of the gastrointestinal mycobiome. Numerous interactions between fungi and bacteria and the complex immune response to gastrointestinal commensal or pathogenic fungi all impact on the pathophysiology of inflammatory bowel disease and other gastrointestinal inflammatory entities such as peptic ulcers. Mycotoxins generated as fungal metabolites contribute to disturbances of gastrointestinal barrier and immune functions and are associated with chronic intestinal inflammatory conditions as well as hepatocellular and oesophagogastric cancer. Systemic and gastrointestinal disease can also lead to secondary fungal infections. Fungal genomic databases and methodologies need to be further developed and will allow a much better understanding of the diversity and function of the mycobiome in gastrointestinal inflammation, tumourigenesis, liver cirrhosis and transplantation, and its alteration as a consequence of antibiotic therapy and chemotherapy.

Conclusions
The fungal microbiota and its metabolites impact gastrointestinal function and contribute to the pathogenesis of digestive diseases. Further metagenomic analyses of the gastrointestinal mycobiome in health and disease is needed.

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INTRODUCTION

The human gastrointestinal (GI) tract is known to contain a variable fungal microbiome, but the phylogenetic characteristics of GI fungal organisms and their specific role as part of the GI ecosystem have not yet been studied extensively. Advances in DNA high-throughput sequencing technology and bioinformatics analysis to date have been mainly aimed at the bacterial microbiome,[1–3] but are now ready to be applied to the analysis of the fungal microbiome. Interactions between fungi and bacteria are common, complex and species dependent, and have dramatic effects on the growth and pathogenesis of microbes.[4] Evidence from human epidemiological and animal studies implicates mycotoxins, generated as fungal metabolites, in GI and liver cancer development and in other chronic GI disorders.[5–7] The GI microbiota engages in a dynamic interaction with the cells of the intestinal innate and adaptive immune system.[8, 9] However, the host immune response to exogenous pathogenic fungi and to potentially harmful indigenous fungal microbiota in the human GI tract is not well understood.

In recent years, the incidence of invasive fungal infections (IFI) has experienced a dramatic increase globally,[10, 11] possibly implying changes in either systemic or local immune responses. Previous studies have mainly focused on pathogenic fungi. Research on the composition and function of the commensal fungal microbiome in health and disease is in its early stages. Some evidence suggests a role for an imbalance between GI commensal fungal and bacterial microbiota or the invasion of host niches by pathogenic fungi or fungal metabolites in inflammatory bowel disease (IBD),[12] peptic ulcers,[13] irritable bowel syndrome (IBS),[14] antibiotic associated diarrhoea (AAD)[15] and chemotherapy-induced enteric disorders,[16] but the potential mechanisms still remain unclear. In this review, we highlight recent advances in this area.

NATURE/CLASSIFICATION OF FUNGI IN THE HUMAN GI TRACT

The human being is a superorganism constituted by the human body and microorganisms. A large number of microbes, including bacteria, archaea, fungi and viruses colonise the skin and mucosal surfaces of the human body. The mean weight of these microorganisms is about 1.5 kg, which is equivalent to that of the liver; the number can be up to 10^{12}–10^{14}, which is 10 times the number of host cells; the number of microbiome genes is 150 times the number of human genes.[2] Decoding of the human genome without knowledge of the microbiome is therefore far from sufficient for a comprehensive understanding of the biological function of the human body. Fungi as eukaryotic microorganism are widely distributed in nature. At present, more than 400 species of fungi associated with human beings have been identified. Previously, fungi have typically been viewed as pathogenic microbes, and their role as commensals has been underappreciated. It is now evident, however, the commensal fungal microbiota is an important part of the human GI ecosystem. Early studies based on culture-dependent methods reported that fungi could be detected in the digestive tract of 70% of healthy adults[17] and that the number of fungi in the human stomach, jejunum, ileum and colon is 0–10^2, 0–10^2, 10^2–10^3 and 10^2–10^6 CFU/mL respectively[17]; most of these are aerobes or facultative anaerobes. Recently, DNA sequencing analysis is widely used for the classification and identification of fungal microbes of plants, animals and various natural environments.[18] From currently available literature, only a few studies have been conducted on the human commensal fungal microbiota using culture-dependent methods or high-throughput DNA sequencing. Research on the fungal microbiome of the human oral cavity and skin is an area of intense investigation.[19, 20]

Fungal microbiota in GI tract

It has previously been thought that, because the normal oesophagus cannot retain food contents, it is either sterile or contains only a few transient microbes from the oropharynx or stomach. However, recent studies based on culture-independent methods showed more diversity and complexity of residential bacterial microbiota in the oesophagus with the representation of nine phyla and up to 160 species.[21, 22] The oesophageal bacterial community differs significantly between the healthy subjects and those with oesophageal disorders.[22, 23] Furthermore, early culture-dependent studies confirmed that *Candida* sp. was the most common fungi in the normal oesophagus. *Candida* oesophagitis is very common in patients with impaired immunity,[24, 25] but the diversity and function of the mycobiome in the oesophagus and its relationship with oesophageal disorders still remain unknown.

It was once believed that gastric acid could kill microbes entering the stomach and that the unique ecological environment of the stomach was not suitable for microbial colonisation or infection. However, several studies using culture-independent methods confirmed that large numbers of acid-resistant bacteria belonging to eight phyla and up to 120 species exist in the stomach,

such as *Streptococcus* sp., *Neisseria* sp. and *Lactobacillus* sp. etc.[26, 27] Furthermore, *Candida albicans* can grow well in highly acidic environments,[28] and some genotypes may increase the severity of gastric mucosal lesions.[29] Recently, a study using bacterial and fungal DNA co-sequencing analysis obtained 19–81 fungal genus-level operational taxonomic units in gastric juice, but only identified two fungal genera including *Candida* and *Phialemonium*.[30] Nevertheless, it is still uncertain whether gastric fungi or bacteria other than *Helicobacter pylori* participate in the pathogenesis of gastric diseases (e.g. in ulcers and cancer, etc.). The real distributions of gastric microbes in various disorders remain to be confirmed, as the microflora in gastric mucosa and stomach contents have not yet been comprehensively studied.

Much less is known about the microbes in small intestine, particularly because collecting samples for such microbial ecology studies is much more challenging. Regardless, it is increasingly clear that the small intestinal microbiota play an important role in IBS, coeliac disease, small intestinal bacterial overgrowth and chemotherapy-induced mucositis. The gut microbiome of small intestinal transplant recipients is altered due to the influence of the donor's commensal bacterial microflora.[31] Moreover, intestinal transplant recipients are often colonised with *Saccharomyces cerevisiae*, *Kluyveromyces waltii*, *Candida* spp., *Cryptococcus neoformans*, *Fusarium oxysporum* and *Aspergillus clavatus*, etc.[32]

While the diversity and function of the colonic bacterial microbiota have been broadly characterised, little is known about the mycobiome in human large intestine and faeces. Previous studies based on culture techniques confirmed that *Candida* spp. are the most common commensal fungi in the intestine. Recently, the metagenomic analysis of 124 individuals reported that only 0.1% of microbial genes in faeces came from eukaryotic or viral origin,[2] which was consistent with previous reports of fungi accounting for only 0.03% of the faecal microbiota.[12] *Candida* spp., *S. cerevisiae* and *Malassezia* spp., etc. were thought to be the predominant commensal fungal species while *Aspergillus* spp., *Mucor* spp., *Cryptococcus* spp., *Rhodotorula* spp., *Trichosporon* spp., *Histoplasma* spp., *Coccidiodes* spp., *Paracoccidioides* spp. and *Blastomyces* spp. were classified as transient microbiota or exogenous pathogenic fungi. It is well recognised that the human intestine could be the largest source of fungaemia when systemic or local mucosal immune functions are disturbed.[33, 34] Moreover, the intestinal mycobiome may contribute to the pathogenesis of several GI disorders, particularly IBD, which will be further elaborated in the following issues.

Influencing factors of GI fungal microbiota

The bacterial microbiota in the human GI tract is affected by age, body mass index, delivery and feeding methods, diet, ethnicity and geographical environments.[35, 36] In addition, pH, oxygen, nutrition and bile acids in the GI tract can influence the composition and function of the microbiota.[37] As with the bacterial microflora, the GI mycobiome could also be affected by these environmental and physiological factors. For example, several fungal species contained in fermented foods and beverages can be also identified in the human GI tract, but whether they are truly indigenous needs to be elucidated. In addition, infants may not harbour any gut mycobiome, but their healthy mothers harbour various intestinal fungi like *Saccharomyces* spp. and *C. albicans*.[38] The intestinal fungal microbiota of an obese individual was investigated by fungal DNA sequencing, which identified 18 species from the *Ascomycota*, *Basidiomycota* and *Chytridiocomycota* phyla, but several fungal species mainly originated from food.[39] Tobacco use is highly associated with the overgrowth of oral and faecal fungal microflora.[40, 41] Furthermore, various iatrogenic factors such as chemotherapy and antibiotic administration could play an important role in the disruption of GI fungal microbiota, which will discussed in the following issues.

METHODOLOGY

Traditional culture-dependent methods for fungal classification and identification include microscopy, growth on selective media and biochemical assays, which examine morphology, growth characteristics and physiological activity of fungi. However, the overall community structure and its spatial and dynamic properties cannot be fully captured using traditional methods due to the fact that most human-associated microorganisms are uncultured. Molecular techniques, highlighted by the complete sequencing of the *S. cerevisiae* genome and 18S rRNA profiling, will greatly improve our understanding of the human mycobiome.

Fungal phylogeny can be reconstructed from the molecular level, such as in the sequences of ribosomal RNA and other highly conserved genes.[42] At present, the DNA sequencing based on the fungal 18S rDNA and internal transcribed spacer (ITS) has been widely applied in fungal microbiota.[43, 44] 18S rDNA sequence has conserved and variable regions, where conserved areas reflect phylogenetic relationships among species, highly variable regions reflect the differences between species. 18S rDNA sequence can be applied to classify fungi at the species level and above. ITS includes ITS1, located between 18S

and 5.8S rDNA, and ITS2, located between 5.8S and 28S rDNA. The variability in ITS1-ITS2 is larger than 18S rDNA and can be used to identify fungi on the species or subspecies level. Co-sequencing of 18S rDNA and ITS may be more suitable for fungal classification. Sequencing results can be compared to existing databases for identification of species present. Moreover, metagenomic sequencing includes all fungal and bacterial genomes in environmental samples, so the co-sequencing of fungal 18S rDNA/ITS and bacterial 16S rDNA sequences may be the optimal and cost-effective method for the investigation of the biodiversity and function of the microbiota which exists in each part of the human body.[30, 45]

FUNGAL MICROBIAL INTERACTIONS

Fungal–fungal interactions

Physical and molecular interactions occur among fungi themselves. For example, the morphological transformation between hypha and yeast forms can influence the adhesion and colonisation of *Candida* sp. in host cells as well as their virulence.[46] In addition, fatty acid metabolites have impacts on the morphological changes of *C. albicans*, as well as its pathogenicity.[47] Glycerol, the small molecular metabolite in biofilm cells of *C. albicans*, is critical for expression of numerous biofilm-regulated adhesion genes during *C. albicans* biofilm formation.[48]

Fungal–bacterial interactions

Published studies on fungal–bacterial interactions to date mainly focus on interactions between *C. albicans* and several pathogenic bacteria in pulmonary, oral and dermatological diseases or in various catheter- and ventilator-related infections.[49] Some troublesome pathogenic bacteria in hospitals, such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Staphylococcus epidermidis*, etc. commonly co−inhabit with *C. albicans*.[49] Polymicrobial infections with *C. albicans* and these pathogens could cause a range of infectious syndromes. Moreover, mixed species biofilms develop through co-aggregation between *C. albicans* and several pathogenic bacteria on the surface of exogenous medical devices and some susceptible sites of the human body, which are thought to be important for the development of infectious disorders.[50] These fungal–bacterial interactions can be antagonistic, synergistic, commensal or symbiotic and influence physical and physiological characteristics such as the mutual morphology, behaviour and survival including response to anti-microbial agents.[51] Known bacterial–fungal interactions are summarised in Table 1.[52–69]

Quorum-sensing molecules are often the basis of the interaction between fungi and bacteria. For example, *C. albicans* can inhibit the virulence of *P. aeruginosa* through farnesol, while *P. aeruginosa* can affect the growth, morphology and virulence of *C. albicans* through the virulence factors pyocyanin and phenazines.[70–72] Fungal morphology can also affect these interactions. For example, *P. aeruginosa* can form a biofilm on the surface of *C. albicans* hyphae and is lethal to them whereas it has no effect on the yeast form of *C. albicans*.[53] Moreover, colonisation of *C. albicans* in rat lungs prior to *Pseudomonas* spp. exposure may increase the incidence of *Pseudomonas*-related pneumonia, because *C. albicans* can inhibit the local host immune response by reducing the production of reactive oxygen species. *Candida albicans* and *Streptococcus* spp. can form a mixed biofilm with anti-microbial antagonism through the polysaccharides on the cell surface of *Streptococcus* spp.[73, 74] In a simulated *in vivo* model, a synergistic effect between *C. albicans* and *Streptococci* increases the invasive ability of *C. albicans* and promotes biofilm formation by *Streptococcus* spp. in the oral and upper GI mucosa.[73] *Acinetobacter* sp. is one of the most common hospital-related pathogens associated with ventilator−associated pneumonia and catheter-related infections. The interactions between fungi and *Acinetobacter* spp. can be antagonistic (e.g. *C. albicans* and *A. baumannii*) or synergistic (e.g. *S. cerevisiae* and *A. baumannii*) (Table 1).[55, 75] It needs to be noted that conclusions drawn from *in vivo* and *in vitro* studies and from mammalian and nonmammalian (e.g. *Caenorhabditis elegans*) models may not be entirely consistent, which could be due to the participation of the host immune system.

Fungal–microbial interactions in GI tract

Interactions among *C. albicans*, *Lactobacillus* spp., *H. pylori* and *Escherichia coli* in the GI tract have been investigated *in vivo* and *in vitro* (Table 1). For instance, the co-existence of *C. albicans* with *H. pylori* could represent synergy in the pathogenesis of peptic ulcers.[76] Furthermore, *Lactobacillus* spp. could inhibit the growth and virulence of *C. albicans* by the production of hydrogen peroxide and organic acids, but could not eradicate *C. albicans*.[47, 61] Also, different *Lactobacillus* strains have different abilities in inhibiting the growth of *C. albicans* in the GI tract.[77] In addition, the effects of *Lactobacillus* spp. on host immune response may be involved in the modulation of fungal virulence.[78, 79] In addition to these strain-specific interactions, interactions between fungi and the bacterial microbiota as a whole have also been

Table 1 | Characteristics of interactions between common fungal and bacterial species

Fungi	Bacteria	Characteristics
Candida sp. *C. albicans*	*Pseudomonas* sp. *P. aeruginosa*	*In vitro*, fungal QS molecule farnesol can inhibit the virulence of *P. aeruginosa*[52] *In vitro*, *P. aeruginosa* can form biofilm on *C. albicans* hyphae[53] *In vitro*, bacterial phenazines and phospholipases can inhibit *C. albicans* growth and virulence[53] *In vivo*, the inhibition of local immunity by *C. albicans* contributes to *Pseudomonas* sp. colonisation[54] *In vivo*, the colonisation of *C. albicans* can increase the risk for *P. aeruginosa*-related pneumonia[54]
Candida sp. *C .albicans*	*Acinetobacter* sp. *A. baumannii*	In *C. elegans* model, *A. baumannii* can inhibit the hyphae and biofilm formation of *C. albicans*[55] In *C. elegans* model, fungal farnesol can reduce the viability of *A. baumannii*[55] *In vitro*, *A. baumannii* OmpA is essential for the attachment to *C. albicans* filaments[56]
Saccharomyces sp. *S. cerevisiae*	*Acinetobacter* sp. *A. baumannii*	In *C. elegans* model, fungal ethanol secretion can enhance *A. baumannii* growth and virulence[75]
Candida sp. *C. albicans*	*Staphylococcus* sp. *S. epidermidis* *S. aureus*	*In vitro*, *S. epidermidis* can adhere to both hyphae and yeast cells of *C. albicans*[57] *In vitro*, bacterial extracellular polymers can protect *C. albicans* from the antifungal agent[57] *In vitro*, fungal farnesol can inhibit *S. aureus* biofilm capability[58] *In vitro*, fungal farnesol can increase the antimicrobial susceptibility of *S. aureus*[58]
Candida sp. *C. albicans*	*Streptococcus* sp. *S. gordonii* *S. oralis* *S. sanguinis* *S. mutans*	*In vitro*, fungal Als proteins mediate aggregation with *S. gordonii* and *C. albicans*[59] *In vitro*, bacterial Ssp proteins mediate the adherence to *C. albicans*[59] *In vitro*, bacterial QS molecule can inhibit the yeast-hyphae transition in *C. albicans*[60]
Candida sp. *C. albicans*	*Lactobacillus* sp. *L. acidophilus* *L. rhamnosus* *L. reuteri*	*In vitro*, bacterial hydrogen peroxide or organic acids can inhibit *C. albicans* growth and virulence[61] *In vivo*, *Lactobacillus* sp. can inhibit the GI colonisation and infection of *C. albicans*[62] *In vivo*, *C. albicans* can suppress *Lactobacillus* sp. regeneration in the GI tract after antibiotic therapy[63, 64]
Candida sp. *C. albicans*	*Helicobacter* sp. *H. pylori*	*In vivo*, co-existence of *C. albicans* with *H. pylori* may indicate the synergism in peptic ulcers[76]
Cryptococcus sp. *C. neoformans*	*Klebsiella* sp. *K. aerogenes*	*In vitro*, *K. aerogenes* can augment *C. neoformans* melanisation and enhance fungal virulence[65]
Candida sp. *C. albicans*	*Salmonella* sp. *S. typhimurium*	In *C. elegans* model, *S. typhimurium* can inhibit the hyphae formation of *C.albicans*[66] *In vitro*, *S. typhimurium* can inhibit *C. albicans* virulence and biofilm formation[66]
Candida sp. *C. albicans*	*Escherichia* sp. *E. coli*	*In vivo* and *in vitro*, *E. coli* endotoxin can enhance the virulence of *C. albicans*[67] *In vitro*, fungal farnesol can increase the susceptibility of *E. coli* to anti-bacterials[68]
Candida sp. *C. albicans*	*Burkholderia* sp. *B. cepacia*	*In vitro*, bacterial DSF can inhibit the germ tube and filament formation of *C. albicans*[69]

QS, quorum sensing; OmpA, outer membrane protein A; GI, gastrointestinal; DSF, diffusible signal factor.

confirmed by some evidence. For instance, the germ-free mouse exhibited an increased susceptibility for the intestinal colonisation of *C. albicans*.[80] Disturbances of the bacterial community in the GI tract promote *C. albicans* colonisation,[81, 82] suggesting that the normal bacterial microbiota of the GI tract have an inhibitory effect against fungal colonisation and invasion. One study in mice showed that the commensal mycobiome could be found in intestinal patches co-habitating with bacterial microbiota, indicating the formation of fungal-bacterial biofilms in GI tract.[83] In addition, several exogenous fungal probiotics, such as *Saccharomyces boulardii*, have been widely used as probiotics in clinical practice since the 1950s in Europe and are strongly recommended for the prevention of AAD.[84] The potential protective efficacy of *S. boulardii* may be due to the regulation of the host immune system and its interaction with pathogenic or normal microbes. Overall, the microbial interactions in the human GI tract are complex and undefined; many interactions remain to be investigated.

MYCOTOXINS

Mycotoxins, small-molecule secondary metabolites of fungi, exhibit diverse structures and can be classified into over 400 species of which about 30 are pathogenic to human beings.[85] The pathogenicity of mycotoxins in humans is mainly caused by the intake of contaminated food. Most food-borne mycotoxins are mainly from *Aspergillus* spp., *Penicillium* spp. and *Fusarium* spp., etc.[85] Some common mycotoxins such as aflatoxins (AF), sterigmatocystin (ST), fumonisin B (FB), ochratoxins A (OTA), trichothecenes and patulin are closely associated with human disease. The toxic effects of mycotoxins associated with the digestive system mainly include carcinogenicity and the disturbance of GI functions.

Carcinogenicity of mycotoxins

Aflatoxins are mainly produced by *Aspergillus* spp. and its subtype AFB_1 is positively associated with hepatocellular carcinoma.[86] High levels of AFB_1 in food and urine are associated with increased risk of hepatocellular carcinoma in the high incidence areas when compared with HBV infection.[5] Moreover, the detection rate of AFG_1 in food is also high in those regions of China in which gastric and oesophageal carcinomas are most common.[87] ST is a late metabolite in the aflatoxin pathway and is also carcinogenic and mutagenic. *In vitro* and animal studies confirmed that ST could promote intestinal metaplasia and may participate in the progression of gastric carcinoma in the presence of *H. pylori*.[88, 89] FB is produced mainly by *Fusarium* spp. The common subtype FB_1 has been demonstrated to cause hepatocellular carcinoma in animal models, but the association between FB_1 and hepatocellular carcinoma in humans has not been confirmed.[90] However, FB_1 may be a potential risk factor for oesophageal carcinoma, but this is still not widely accepted.[91] OTA is produced mainly by *Aspergillus* spp. and *Penicillium* spp. The kidney is the primary target organ for OTA, so the renal toxicity is obvious; in addition to nephrotoxicity, liver toxicity, carcinogenicity and genotoxicity were shown in animal models.[92] Trichothecenes, represented by deoxynivalenol (DON), nivalenol (NIV) and T-2 toxin, are produced mainly by *Fusarium* spp. The detection rate of DON and NIV in contaminated food in the Linxian and Cixian areas of China with a high incidence of gastric and oesophageal carcinoma is much higher, and feeding with contaminated grains in these areas could induce benign and malignant tumours in mice.[93] In total, the association between AF and hepatocellular carcinoma has been confirmed, but the carcinogenicity of the other mycotoxins on human beings is still controversial. Epidemiological, *in vivo* and *in vitro* studies should be conducted to draw definitive conclusions.

Effects of mycotoxins on intestinal functions

The GI tract, as the primary targeting organ of mycotoxins, is exposed directly to mycotoxins with a higher concentration than other tissues and organs, which can affect the regeneration, proliferation, differentiation and repair of intestinal epithelial cells.[94] Some mycotoxins, such as FB_1, OTA and DON, could reduce the expression of zonula occludens protein and increase intestinal mucosal permeability, thus damaging the barrier functions of intestinal epithelial cells and inducing bacterial translocation.[6, 95] Moreover, mycotoxins can influence the immune system of the GI tract.[94] Mycotoxins could trigger mucosal immunoregulatory mechanisms such as the secretion of mucus, anti-microbial peptides and immunoglobulins, and directly induce inflammation by inducing intestinal epithelial cells to secrete chemotactic factors and pro-inflammatory cytokines.[96–98] Mycotoxins may be associated with chronic inflammation of the intestine in genetically susceptible patients with IBD or coeliac disease, but further studies should be conducted to confirm this relationship.[97] Interestingly, some studies based on culture methodology showed that mycotoxins can change the composition of intestinal bacterial communities. For example, the long-term exposure of DON or T2 toxin can increase intestinal colonisation by aerobes.[94] But the definite disturbance of human GI fungal and bacterial microbiota induced by mycotoxins still remains largely unknown.

THE IMMUNE RESPONSE TO FUNGI

Fungal pathogens elicit innate immune responses at mucosal epithelial cell surfaces of the GI, respiratory and genitourinary tracts and in the skin.[9] Various fungal cell wall components, including β-glucans, zymosans, mannans, chitin, DNA and RNA are the main sources of pathogen-associated molecular patterns (PAMPs) that are recognised by host cells. Immunity to fungi critically requires pattern recognition receptors (PRRs) on human host cells to recognise and trigger innate and adaptive immune responses: these PRRs include Toll-like receptors (TLRs), C-type lectin receptors (CLRs), NOD-like receptors (NLRs), galectin family and retinoic acid-inducible gene-I-like receptors (RLRs). These PRRs are mainly present in host phagocytic cells including monocytes, macrophages and neutrophils, as well as some nonphagocytic cell types, such as epithelial and endothelial cells, which could contribute to the anti-fungal innate immune

response through phagocytosis and direct pathogen killing. The immune responses mediated by PRRs on related host cells include fungal binding and phagocytosis, induction of anti-fungal effector mechanisms and the production of various soluble mediators including defensins, cytokines, chemokines and some inflammatory lipids. In addition, PRRs also direct and modulate the development of adaptive immunity, particularly the TH1 and TH17 responses induced by dendritic cells. We should note that multiple PRRs could be stimulated by fungal PAMPs in different combinations depending on the fungal species and on the host cell types. Challenges exist in the field of the human host immune response to fungi, which include the control of inflammation leading to tolerance, the basis of immune regulation and dysregulation, and the link between PRRs polymorphisms and fungal susceptibility in the context of various digestive diseases. In-depth studies of the complex cross-talk mechanisms present in the process of host immunity to fungi, especially of the GI mucosal immune response to commensal fungi should be conducted.

Main fungal-related PAMPs and PRRs

C-type lectin receptors, including Dectin-1, Dectin-2, DC-SIGN, mannose receptor and mannose-binding lectin, play a central role in the recognition and shaping of immune responses to fungal pathogens.[99] The associated fungal PAMPs recognised by various CLRs are shown in Table 2. Dectin-1, for example, is the key CLR-recognised fungal β-glucan.[99] Dectin-1 gene deletion can enhance the host's susceptibility to fungi and thus increase the fungal colonisation and dissemination in animal models.[100, 101] The two main signalling pathways involved in the activation of Dectin-1 are spleen tyrosine kinase-caspase recruitment domain-containing protein 9 (CARD9) and serine/threonine protein kinase Raf-1, which may have synergetic function and induce the activation of NF-κB through cross-talk mechanism.[102] Moreover, a recent study in a murine colitis model reported that CARD9 could influence the colonisation of fungi by mediating intestinal mucosal immune responses.[103] In addition, Dectin-1 could mediate fungal immune response through the synergistic collaboration with TLRs.[99, 104] Polymorphism in Dection-1 has been linked to host susceptibility of several fungi species, and fungal colonisation and infection.[105–107] Recently, one study has examined how intestinal commensal fungi interact with the host immune system through Dectin-1 in a murine model of colitis.[83]

Toll-like receptors, particularly TLR2, 3, 4, 6, 7 and 9, as the members of the best-characterised PRRs families, are closely associated with fungal immune recognition and response. Both the exogenous pathogenic fungi (e.g. *Aspergillus* sp., *Cryptoccocus* sp. and *Coccidoides* sp.) and the commensal fungi (e.g. *Candida* sp.) are recognised by TLRs. The contribution of individual TLRs may vary depending on the fungal species, fungal morphotypes, route of infection and receptor co-operativity. With regard to human studies, a polymorphism in TLR4 is associated with increased susceptibility to pulmonary aspergillosis and bloodstream candidiasis, and a polymorphism in the promoter of TLR9 is associated with allergic bronchopulmonary aspergillosis.[108] TLR1 and TLR6 gene polymorphisms are associated with the susceptibility of invasive aspergillosis in patients with haemopoietic stem cell transplantation (HSCT).[109]

NOD-like receptors also contribute to the anti-fungal immunity intracellularly, although fungal PAMPs for

Table 2 | Main PRRs associated with host recognisation for fungi

Fungal PAMPs	PRRs	Characteristics of inflammatory response
β-glucans	Dectin-1	Phagocytosis
Mannans	Dectin-2	Defensins secretion
N-linked mannans	DC-SIGN	Chemokines secretion
N-linked mannans /α-glucans/chitin	MR	Cytokines secretion
Mannose/mannans	Mincle	
Glycans	Langerin	
Mannose	MBL	
β-glucans /PLMmannans /zymosans	TLR2	Phagocytosis
dsDNA	TLR3	Defensins secretion
O-linked mannans/proteases	TLR4	Chemokines secretion
Zymosans/PLM	TLR6	Cytokines secretion
RNA	TLR7	
CpG DNA	TLR9	
/	NLRs	Inflammasomes formation Cytokines production
β-mannosides	Galectins	Fungal killing directly Cytokines production
β-glucans	CR3	Phagocytosis Cytokines secretion
β-glucans	CD36	Phagocytosis Chemokines secretion Cytokines secretion

PRRs, pattern recognition receptors; PAMPs, pathogen-associated molecular patterns; CLRs, C-type lectin receptors; MR, mannose receptor; MBL, mannose-binding lectin; PLM, phospholipomannans; TLRs, toll-like receptors; NLRs, NOD-like receptors; CR3, complement receptor 3; /, unclear.

NLRs are not yet clear. NLRs could induce the production of IL-1β and IL-18 through the formation of inflammasomes, which are essential for protective anti-fungal immunity, particularly for driving the development of anti-fungal TH17 and TH1 responses.[110, 111] The NLRP3 inflammasome plays an important role in the regulation of host defence against microbial pathogens and intestinal homoeostasis,[112] the damage of which could impair the integrity of intestinal epithelial cell,[113] enhance the colonisation of *C. albicans*, and then promote the formation of granulomas in the GI tract.[114] In addition, some evidence showed that the dysbiosis mediated by NOD2 could induce colitis and colorectal cancer,[115] NOD2 gene mutations are associated with increased susceptibility to Crohn's diseases (CD) as well as increased levels of circulating anti-*S. cerevisiae* antibodies (ASCAs).[116]

ROLE OF FUNGI IN PATHOGENESIS OF GI DISORDERS

Digestive system-related fungal infections

The host immune system can tolerate the colonisation of commensal fungi in the GI tract, but defend against fungal invasion. However, this balance is disturbed in systemic immunosuppressive states including, for example, the acquired immune deficiency syndrome (AIDS), leukaemia and HSCT, solid organ transplantation and immunosuppressant therapy, anti-microbial and steroid treatments, total parenteral nutrition, iatrogenic catheters and mechanical ventilation, malignant tumours, chemoradiotherapy and diabetes mellitus, all of which are associated with enhanced fungal colonisation and infection in the GI tract. Similarly, localised insults in the digestive system, such as GI mucosal lesions and surgical procedures, can also lead to GI fungal infection. In addition, GI fungal infection is reported even among those patients with normal immune status. Digestive system-related fungal infections may be induced by both commensal opportunistic fungi and exogenous pathogenic fungi. The IFI in different GI sites have their special clinical features, which are often accompanied by various severe diseases. Although IFI associated with digestive diseases are less common, they can induce fatal outcomes due to less specificity of related symptoms, signs, endoscopic and imaging manifestations, and the poor treatment options. Further works should focus on the early diagnosis and treatment for the GI fungal infections, and the proper application of anti-fungal drugs.

Among oesophageal IFI, *Candida* sp. is the most frequently identified species in patients with immunodeficiency conditions, especially AIDS. Other unusual pathogenic fungi such as *Cryptococcus* sp., *Aspergillus* sp., *Mucor* sp., *Paracoccidioides* sp. and *Saccharomyces* sp. have also been isolated. Severe oesophageal IFI could cause oesophageal stricture and perforation, and often coexists with reflux oesophagitis, oesophageal cancer and AIDS.[117] Oesophageal fungal infection is one precipitating factors for oesophageal cancer and can cause local chronic inflammation and result in the proliferation of epithelial cells and enhanced susceptibility to carcinogens. On the other hand, the metabolites produced by fungi could directly act on the oesophageal epithelial cells, thus resulting in carcinogenesis.

Candida sp. is also the most frequently identified species among patients with gastric IFI. We also need to recognise the emergence of gastric mycosis caused by *Basidiobolus* sp., *Mucor* sp., *Cryptococcus* sp., *Histoplasma* sp., *Aspergillus* sp., *Paracoccidioides* sp. and *Cryptococcus* sp. Gastric IFI is often characterised by the abdominal pain and vomiting and with the endoscopic characteristics including gastric giant and multiple ulcers, stenosis, perforation and fistula. For example, gastric ulcers combined with entogastric fungal infection, characterised by deep, large and intractable ulcers,[118] were reported as early as the 1930s.[119] More than 50% of patients with gastric ulcers present with gastric fungal colonisation, which often appears among the elderly population with low gastric acid.[28] Moreover, the fungal infection could occur in gastric carcinoma and residual gastritis, which may be due to the injury of gastric mucosa barrier functions, the long-term use of antibiotics, chemoradiotherapy, the stomach tumour itself and the surgical procedures.[120] In addition, various systemic immunosuppressive disorders can impair the local immunity of gastric mucous, and gastric fungal infection is not uncommon.[30, 121]

Liver IFI is mainly induced by the systemic immunosuppressive state and is one manifestation of systemic disseminated fungal infection. Patients with liver IFI often present with abdominal pain, obstructive jaundice, abnormal hepatic enzymes, hepatosplenomegaly and liver abscess. Pathogenic fungi including *Mucor* sp., *Candida* sp., *Aspergillus* sp., *Cryptococcus* sp., *Histoplasma* sp., *Coccidioides* sp., *Malassezia* sp. and *Paracoccidioides* sp., were reported to cause local infection of liver.[122–124] On the other hand, some severe liver disorders can also cause the systemic disseminated fungal infection. For example, IFI is one of the major complications of patients with chronic severe hepatitis, liver failure and decompensated cirrhosis; higher hepatitis B virus DNA

levels may be an independent risk factor.[125] Furthermore, IFI is more common in liver transplant recipients and is associated with increased morbidity and mortality following liver transplantation.[126, 127] The incidence of IFI after liver transplantation is up to 15–42% and the fatality rate is 40–80%, which is higher than that of acute rejection, renal failure and virus infection.[126–128] *Candidas* sp. are the most common pathogenic fungi after liver transplantation, and *Aspergillus* sp., *Cryptococcus* sp. show a tendency to increase.[127, 128] The higher incidence of IFI after liver transplantation is associated with end--stage liver diseases, operative procedures, hyperglycaemia, post-operative immunosuppressants, and hormone and antibiotics treatments.[128] Furthermore, one study reported a higher intestinal fungal biodiversity among patients with chronic HBV infection, but *Candida* sp. and *S. cerevisiae* were the only dominant fungi that could be cultured. Importantly, the fungal diversity and detection rate was positively correlated with disease progression.[129] It is still not clear whether and how the intestinal mycobiome contributes to the incidence of IFI among patients suffering from the end-stage liver diseases and liver transplantation.

The overgrowth and colonisation of fungi in intestine can lead to diarrhoea. Although the human intestine is the largest source of fungaemia in the immunocompromised host, IFI with intestinal involvement is an uncommon but serious complication.[130, 131] Several fungal species such as *Saccharomyces* sp., *Candida* sp., *Mucor* sp., *Basidiobolus* sp., *Aspergillus* sp., *Histoplasma* sp., *Mucor* sp., *Cryptococcus* sp., *Geotrichum* sp. and *Actinomyces* sp. could cause small intestinal and colonic IFI. Severe intestinal IFI could produce the clinical presentations of anorexia, abdominal pain, watery diarrhoea, lethal bowel necrosis and infarction, GI bleeding, ulcers and perforation. Importantly, there have been attempts to account for the possible role of fungi in the pathogenesis of some intestinal diseases such as IBD and IBS, which, while plausible, remains unproved. Moreover, intestinal fungi are closely associated with the intestinal disorders after chemoradiotherapy and antibiotic treatment, which are also the major concerns of gastroenterologists.

Fungal interactions with *H. pylori* in peptic ulcers

Candida-associated peptic ulcers are more common than previously considered and are characterised by the ulcer's delayed healing process and even perforation. As with *H. pylori*, *Candida* sp. may play an aetiologic role in peptic ulcers, as it can be detected in the *H. pylori*-negative patients with peptic ulcers.[132] But other views suggest that fungi alone cannot play a pathogenic role as they colonise only gastric epithelium,[76] and the presence of fungi in the stomach is a secondary phenomenon of peptic ulcers.[133] One study showed that 66.7% of gastric disorder patients with *Candida* sp. in the gastric mucosa were co-colonised with *H. pylori*.[76] Although causation vs. secondary colonisation was never actually examined in these studies, the co-existence of *C. albicans* with *H. pylori* may indicate synergism in pathogenesis of peptic ulcers, and hypha formation by *Candida* sp. could contribute to the mechanism of ulcer perforation.[76] Several studies found the intracellular occurrence of *H. pylori* in *Candida* sp. and confirmed that *H. pylori* in the vacuole *Candida* sp. could be viable and express several *H. pylori*-specific proteins such as VacA, urease and peroxiredoxin.[134, 135] Accordingly, the vacuole of *Candida* sp. as the reservoirs and vehicles of *H. pylori* could offer the essential nutrients for *H. pylori* survival and multiplication, and protect *H. pylori* against hostile gastric conditions. These interactions between *H. pylori* and *Candida* sp. may play a crucial role in their gastric colonisation, but whether they also contribute to the pathogenesis of peptic ulcers has not been explored.

Role of fungi in GI and liver cancers

According to the limited literature, the carcinogenicity of fungi is mainly due to the long-term intake of exogenous mycotoxins in contaminated food. As discussed earlier, mycotoxins may be involved in the development of hepatocellular carcinoma, gastric and oesophageal carcinoma.[86, 87] Furthermore, the fungal infection and colonisation in the stomach is common in patients with gastric cancers. *Helicobacter pylori* infection is the definitive risk factor for gastric cancers,[136] so interactions between *Candida* sp. and *H. pylori* may play an aetiologic role in the gastric carcinogenesis. We are just beginning to understand that the disturbance of intestinal microbiota could play a significant role in colorectal cancer,[137] characterised by the increased abundance of sulphate-reducing bacterial and the lower proportions of butyrate-producing bacteria.[138] Moreover, butyrate is a bacterial metabolite that provides fuel for the colonic mucosa and possesses anti-inflammatory and anti-proliferative properties, the suppression of which could increase the risk of colorectal cancer.[139] However, it remains to be determined whether the disturbance of the indigenous GI mycobiome and their metabolites contributes to the tumourigenesis. Certainly, it should also be noted that the chronic inflammation of GI epithelial cells

generated by dysbiosis contributes to the neoplastic transformation of GI tract in combination with genetic susceptibility of the host.

Role of fungi in the pathogenesis of IBD

Regarding the intestinal microbiota, most studies in this area have focused on the composition and function of bacterial microbiota and its relationship with several metabolic diseases (obesity, diabetes, metabolic syndrome) and intestinal disorders including IBD, IBS, colorectal cancer.[2, 3, 140] The intestinal bacterial microbiota and related metabolites can work together with the host genetic susceptibility and host systemic and local mucosal immune response to contribute to the pathogenesis of IBD.[2, 141–144] Only a few investigations on intestinal fungal microbiota and its relationship with IBD have been conducted. Much evidence has shown that fungi and their communities may be involved in the pathogenesis of IBD, especially CD.[145] The colonisation of *C. albicans* can exacerbate intestinal inflammation in a murine colitis model, and obvious improvement can be observed after anti-fungal treatment.[146] ASCAs as one of the serological markers for CD could also be induced by *C. albicans*.[147, 148] Moreover, *C. albicans* can be isolated from the intestine more frequently in CD patients and their healthy relatives, but the positive association between ASCAs level and the amount of intestinal *C. albicans* in CD still remains controversial.[149] In addition, inhibition of IL-17A by secukinumab is ineffective in active CD patients,[150] which may be linked to *C. albicans* thriving in the gut induced by loss of control by IL17.[151] Large amounts of *Candida* sp. can also be detected in the faeces or intestinal mucosa among patients suffering from ulcerative colitis,[146, 152] and the clinical symptoms and intestinal inflammation may be improved after the anti-fungal treatments.[146] There were dominant differences in fungal community structure related to IBD compared with that of controls. Fungi sequences could be detected in all IBD patients' colonic mucosa, and the diversity of the intestinal mycobiome was clearly increased among IBD patients, but the proportion of the mycobiome in the whole intestinal microbiota was very low.[12] Further study needs to be conducted on whether the change in fungal community structure is secondary to the imbalance of intestinal bacterial community or an independent pathogenic factor in IBD. Recently, a study connected intestinal fungal microbiota with the host immune system through Dectin-1 in a murine model of colitis, in support of a possible fungal aetiology for IBD.[83]

Role of fungi in the pathogenesis of IBS

Epidemiological and clinical data support the role of GI microbiota in the pathogenesis of IBS. The disturbances of GI bacterial microbiota and their metabolites (e.g. butyrate, acetate and propionate; CH_4 and H_2 gases) have been observed in IBS patients.[153] In addition, IBS symptoms which begin with a GI infection is known as post-infectious IBS.[154] Pathogenic bacteria, viruses and parasites are involved in the occurrence of post-infectious IBS, but it is not clear whether pathogenic or commensal fungi in GI tract are responsible for IBS symptoms. In any case, the dominant symptoms of *Candida*-associated infectious diarrhoea and those of the so-called 'candida syndrome' are similar to IBS associated symptoms. Moreover, several studies reported that the fungi-containing foods combined with intestinal fermentation disturbances could be related to the IBS-related symptoms and food intolerance. Food intolerance may then be a consequence of the bacterial microbiota disturbance and the overgrowth of *C. albicans* in GI tract.[155] However, some studies demonstrated that the overgrowth of *C. albicans* was not the cause of food intolerance in patients with IBS and there was no conclusive link between the overgrowth of *C. albicans* in intestine and the symptoms of IBS.[156] In addition, some evidence showed that fungal products and antigens could cause IBS symptoms in sensitised patients.[14] For example, components (e.g. glycoproteins) and products (e.g. alcohol) of *C. albicans* which are potentially immunogenic and allergenic could provoke mast cell-mediated hyperreactivity and disturb intestinal immunity and barrier function. These pathogenic mechanisms are similar to the pathogenesis of IBS. Regardless, there remains little evidence to support the possible involvement of fungi in the aetiology of IBS.

Fungal enteritis secondary to chemoradiotherapy

Invasive fungal infections have emerged as a significant problem in patients with solid tumours or haematological malignancies undergoing chemoradiotherapy and HSCT procedures with an incidence of 10–15% and the mortality rate of 50–90%.[157] The predominant causative fungal species include yeasts such as *C. albicans* as well as other Candida species (e.g. *C. glabrata*, *C. parapsilosis*, *C. krusei* and *C. tropicalis*), and the filamentous fungi such as *Aspergillus* sp. The frequency of isolation of *Cryptococcus* sp., *Scedosporium* sp., *Fusarium* sp., *Zygomycetes* sp. and *Trichosporon* sp. is currently rising. A survey in adult cancer patients described a shift in epidemiology of candidiasis, characterised by significantly

lower rates of candidemia with non-*C. albicans* sp. (e.g. *C. glabrata* and *C. krusei*) in patients with solid tumours compared with those with haematological malignancies.[158] However, for intestinal IFI, there are fewer comprehensive data for patients with GI solid tumours, although it appears that the species distribution is more consistent with that of general hospital patients.[159, 160] Furthermore, among cancer patients who have received chemoradiotherapy, fungal infection and fungal colonisation mainly occurred in the oral cavity and lung. Invasive fungal enteropathy is an unusual presentation, which most often occurs in the setting of disseminated infection and the true incidence of which may be underestimated. The diagnosis of enteric fungal infection can be delayed because of its nonspecific presentation as well as the lack of characteristic imaging. Clinicians need to make a decision on whether to prevent or treat IFI in cancer patients receiving chemoradiotherapy.

Mucositis following chemotherapy is a major oncological problem which results in GI ulcers and symptoms such as nausea, vomiting, dysphasia, abdominal pain, diarrhoea and constipation.[161] More than 50% of patients receiving 5-fluorouracil or irinotecan develop either GI or oral mucositis, but GI mucositis has not been well defined and the underlying mechanisms of the condition are yet to be established. From the little research conducted, it is likely that there could be a potentially important relationship between commensal microbiota and the subsequent development of chemotherapy-induced mucositis.[16, 162] The abundance of the certain commensal bacterial populations is significantly influenced by chemotherapy, with a decrease in *Bifidobacterium* sp., *Lactobacillus* sp., *Veillonella* sp. and *Faecalibacterium* sp. and an increase in *Enterococcus* sp.[163] Dysbiosis of the intestinal bacterial microbiota could contribute to the development of chemotherapy-induced mucositis by influencing the inflammatory process and oxidative stress, intestinal permeability, resistance to harmful stimuli and epithelial repair, the intestinal mucus layer, and the activation and release of immune effector molecules. Theoretically, chemoradiotherapy could aggravate the GI mucosal lesion, resulting in mucositis, as the mucosal barrier is the portal of entry that allows for invasion of fungi. It is well recognised that the overgrowth of GI fungi could be observed among cancer patients undergoing chemoradiotherapy, but the influence of chemotherapy on GI commensal fungal microbiota remains poorly described, and whether fungi in the GI tract play an important role in chemotherapy-induced mucositis still remains unclear.

Role of fungi in AAD

It is confirmed that prolonged antibiotic treatment has dramatic long-term effects on the commensal GI microbiota, which could disturb the homoeostasis of GI bacterial communities, and thus pre-dispose to the fungal colonisation and infection. Fungal pathogens may play a relevant, but secondary role compared to pathogenic bacteria such as *Clostridium difficile*. The effect of broad-spectrum antibiotics on the intestinal microbiota is characterised by the decrease in protective bacterial populations and the increase in fungi. However, the role of fungi in AAD is still controversial.[15, 164] It has been reported that the faecal concentrations of *Candida* sp. of $\geq 10^5$ CFU/mL can cause diarrhoea in patients receiving antibiotic therapy, but which $<10^5$ CFU/mL do not. It has also been hypothesised that fungal virulence factors such as phospholipases and secreted aspartyl proteinases may contribute to AAD, but some suggested that fungi and their metabolites were the result of antibiotic treatment or diarrhoea rather than a cause of AAD.[164, 165]

On the other hand, the GI microbiome also plays a significant role in the recovery from antibiotic treatment. Several animal studies documented that treatment with broad-spectrum antibiotics could induce the colonisation and overgrowth of *C. albicans* in the caecum and stomach; most importantly, the colonisation of *C. albicans* could also modulate the antibiotic recovery of GI microbiota, antagonising *Lactobacillus* sp. and facilitating *Enterococcus faecalis* colonisation. *Candida albicans* colonisation in the stomach can antagonise the intragastric colonisation of lactic acid bacteria after antibiotic treatment, but can make *E. faecalis* colonise more easily.[63] In addition, the diversity of the bacterial microbiota in the intestine of mice decreased after antibiotic treatment, and the intestinal colonisation of *C. albicans* contributed to the recovery of the bacterial diversity, but resulted in the reduction in *Lactobacillus* sp. and the increase in *E. faecalis*.[64] On the whole, the research to date has focused on the disturbance of bacterial microbiome after antibiotic treatment; however, the GI fungal communities indeed influence the reassembly of GI bacterial microbiota after treatment with broad-spectrum antibiotics.[63, 64] We should begin to highlight the fungal–bacterial interactions in these processes.

CONCLUSIONS

Until now, the influence of symbiotic fungal community on human health and disease has been ignored. We have little knowledge about the distribution of the fungal

microbiota and its function in the human digestive tract. Further studies are needed to explain (i) the cross-talk interactions between fungi and bacteria at the species or community level in the human GI tract, particularly the formation of mixed biofilms and their role in the maintenance of normal physiological function and pathogenicity; (ii) the influence of pathogenic fungi and the commensal fungal microbiota on host systemic immune and local GI mucosal immunity; (iii) the characteristics of invasive fungi associated with various digestive diseases; and (iv) the involvement of fungi in the host energy homoeostasis and metabolic functions. The study of the microbial community has entered the era of metagenomics. Further research should be conducted to investigate the diversity and abundance of the faecal- and GI mucosa-associated mycobiome, and its potential pathogenic role in different digestive diseases such as IBD, peptic ulcers and GI cancers. Whether the alteration of the mycobiome in GI tract is secondary to an imbalanced bacterial microbiota or an independent aetiologic factor needs to be investigated. At present, the co-sequencing of fungal and bacterial DNA may be the optimal and cost-effective method for the metagenomics studies. The application of metagenomics combined with metabonomics, transcriptomics and proteomics, as well as human genome-wide association studies, will contribute to our understanding of the role of the fungal microbiota in human GI health and disease.

AUTHORSHIP

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Author contributions: ZKW performed a literature search, analysed the data and wrote the manuscript. This article was reviewed and amended by YSY, ATS, GS and LHP. All authors approved the final version of the manuscript.

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