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Human breast milk: A promising treatment for necrotizing enterocolitis

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ABSTRACT

Necrotizing enterocolitis (NEC) is a severe gastrointestinal disorder occurring in newborns, with a mortality rate ranging from 20 % to 30 %. The existing therapeutic approaches for NEC are limited in their effectiveness. Various factors contribute to the development of NEC, including disruption of barrier function, dysregulation of the intestinal immune system, and abnormal colonization of the intestinal microbiota. Researchers have shown considerable interest in exploring the therapeutic potential of the constituents present in human breast milk (HBM) for treating NEC. HBM contains numerous bioactive components, such as exosomes, growth factors, and oligosaccharides. However, the precise mechanisms by which HBM exerts its protective effects against NEC remain incompletely understood. In this study, our objective was to comprehensively review the bioactive substances present in HBM, aiming to facilitate the development of novel therapeutic strategies for NEC.

1. Introduction

Necrotizing enterocolitis (NEC) is a devastating condition commonly observed in premature infants [1]. The current treatment options for NEC include fasting, gastrointestinal decompression, intravenous nutrition support, and anti-infection therapy, among others. Despite various strategies being investigated, the available treatments are inadequate, leading to a persistently high mortality rate. Furthermore, infants affected by NEC face both short-term (such as strictures, intestinal dysmotility, sepsis, and perforation) and long-term (including neurodevelopmental delay, intestinal failure, and failure to thrive) complications related to bowel function [2].

The development of NEC is influenced by multiple risk factors, including low birth weight, premature birth, prolonged parenteral feeding, formula feeding, smoking, pregnancy-induced hypertension, gestational diabetes, and preeclampsia, among others [3–5]. Currently, there is no consensus on a universally accepted medical treatment for NEC. Interestingly, researchers have highlighted the significant role of human breast milk (HBM) in promoting the health of premature infants [6]. Numerous studies have demonstrated a lower incidence of NEC in preterm infants fed with HBM compared to formula-fed infants [7,8]. Furthermore, HBM has been shown to possess various beneficial prop - erties, such as immunomodulation, regulation of intestinal microbes, and repair of intestinal barrier function [9–11]. It is widely acknowl - edged that the components present in HBM, including cells, exosomes, oligosaccharides, growth factors, and other bioactive constituents, are specifically tailored to meet the needs of neonates [12–14]. Consequently, HBM holds considerable importance in the treatment of NEC in neonates. Fig. 1 depicts the focus of the present study, which involves a comprehensive review of the existing knowledge regarding the thera - peutic effects of HBM components on NEC. Additionally, this study re- views the mechanisms of action of breast milk in NEC, as reported in previous studies (Table 1). It is anticipated that the findings of this study will provide valuable insights into the role of HBM in the development of novel anti-NEC treatments.

2. HBM-derived exosomes

2.1. HBM-derived exosomal contents

Exosomes are generated from intraluminal vesicles and are released into the luminal space through exocytosis by merging with the cellular membrane [15]. These nano-sized particles (30–150 nm) can be secreted by various cell types, and contain bioactive constituents, such as messenger RNAs (mRNAs), proteins, enzymes, and microRNAs (miRNAs) [16,17]. Exosomes are present in different body fluids, including

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breast milk, saliva, and blood [18–20]. Over the past three decades, extensive research has been conducted on exosomes in diverse fields such as immunology, physiology, and metabolism [15,21]. Breast milk exosomes exhibit remarkable bioactivity, making them a promising approach for treating NEC.

Different types of human extracellular vesicles have shown protec - tive effects on intestinal epithelial cells (IECs) [22,23]. To investigate the therapeutic potential of HBM-derived exosomes in NEC treatment, researchers utilized an in vitro organoid model that mimics the intesti - nal microenvironment. [24]. They also examined the impact of lactation duration on the efficacy of HBM-derived exosomes [25,26]. In an in vitro model, the administration of colostrum-derived exosomes (days 1–5 post-birth), transitional-derived exosomes (days 6–14 post-birth), and mature-derived exosomes (days >15 post-birth) demonstrated a reduc - tion in inflammatory injury [25]. Among these exosome subtypes, colostrum was found to be the most effective in combating pro-inflammatory responses [27]. Moreover, in vivo, models showed that intraperitoneal or enteral administration of HBM-derived exosomes reduced the incidence of NEC in rat pups [26]. Researchers evaluating intestinal pathology in rats reported that HBM-derived exosomes pre- served villi integrity and promoted overall cell growth [28]. Gabrielsson et al. found that early HBM had a higher abundance and purity of exo- somes than mature HBM [29]. Pierro et al. demonstrated that exosomes derived from rat milk (RM-exosomes) enhanced viability, growth, and activity of IECs [30]. Martin et al. observed that HBM-derived exosomes exhibited resistance against H_2O_2-induced oxidative stress (OS) [31].

In intestinal cells, HBM-derived exosomes consist of various com- ponents, with miRNAs being extensively studied. MiRNAs are small non-coding RNAs (ncRNAs), approximately 22 nucleotides in length. They primarily function within exosomes by binding to the 3 untranslated region (3 UTR) of target mRNAs, resulting in target translation inhibi - tion or degradation [32]. Recent research indicated that HBM-derived exosomes could ameliorate NEC progression through the miR-148a- 3p/p53/SIRT1 axis. Agomir therapy targeting miRNAs could poten - tially serve as a novel treatment approach for NEC [17]. Additionally, HBM-derived exosomes aid in protecting epithelial tight-junction pro- teins ZO-1, claudin, and occludin against inflammatory assaults [23].

2.2. *HBM-derived exosomes in cell-free therapy*

HBM contains a variety of bioactive components, including cells, exosomes, growth factors and oligosaccharides, among others. [33]. HBM is particularly rich in different types of cells, such as immunolog - ical cells, stem cells, and epithelial cells [34]. While the immunological benefits of breastfeeding are well-established, there is still a need to explore the specific roles of certain immune cells, particularly effector memory cells and innate immune cells like macrophages, in the context of NEC. Although breast milk has been shown to prevent the develop - ment of NEC, there have been no direct human studies utilizing breast milk stem cells for NEC. However, in vitro investigations have demon - strated that fresh HBM is a valuable source of self-renewing stem cells capable of differentiating into cells from all three germ layers [35–37]. While the precise functions and fate of HBM stem cells are not fully understood, their active involvement in growth, regeneration, and po- tential reparative effects on injured IECs in NEC is plausible.

Despite the potential of stem cell therapy as a treatment option for

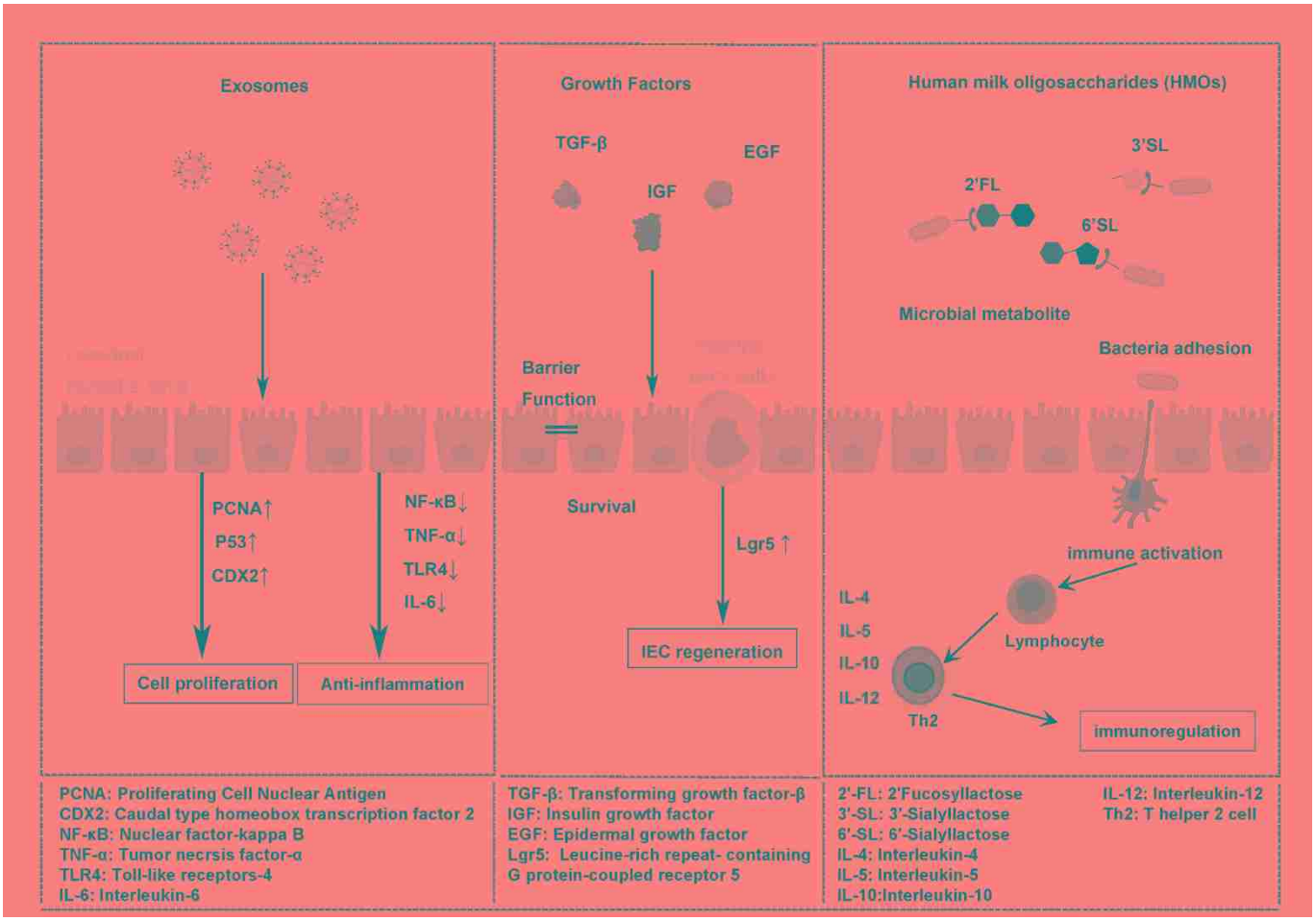

Fig. 1. Potential mechanisms of HBM to prevent NEC in preterm infants.

Table 1
Protections and mechanisms of HBM in NEC.

Component	Model	Role in NEC	Mechanism	Reference
Exosomes	In vitro & inflammatory	Attenuates	miR-148a-3p/p53/SIRT1	[17]
	In vitro & In vivo	Restoring intestinal tight-junction proteins	TLR4	[23]
	In vitro & In vivo	Proliferation and reduce cell apoptosis	/	[24]
Growth factors	EGF In vivo	Attenuates inflammatory response	SP1	[63]
	Optimizes epithelial function		Wnt	[61]
	In vivo	Increases proliferation of intestinal epithelium	/	[62]
	TGF- In vitro &	Attenuates inflammatory response	NF- B	[69]
	IGF In vitro	Promote intestinal maturation	/	[72]
	Ex vivo	Neonatal growth, promote angiogenesis process	/	[73]
HMOs	Ex vivo	Maintain gut microbiome	/	[94]
	Ex vivo	Regulate the responses of immune	/	[90]
	Anti-apoptosis, anti-inflammation, and antioxidative stress in intestinal epithelial cells		/	[109]

immuno - globulins in the gastrointestinal tract and mammary gland of newborns, and aiding in the repair of the intestinal mucosa [49]. It also plays a role in inducing oral tolerance. EGF, another abundant peptide in breast milk, is crucial for maintaining intestinal barrier function, enhancing the activities of intestinal enzymes, and facilitating nutrient transport to the regulation of

NEC, there are limitations associated with stem cells, including altered cell phenotype, limited self-renewal ability, and reduced differentiation potential upon multiple passages in in vitro culture [38]. Recent research has shown that exosomes are highly effective in reducing inflammation, preserving intestinal function, and enhancing gut barrier integrity [39,40]. Compared to stem cell-derived exosomes, HBM- derived exosomes are readily accessible and can be administered orally [5]. Studies have reported that colostrum-derived exosomes exhibit the highest efficacy, and the pasteurization of HBM does not diminish their effectiveness [41]. Exosomes play a critical role in regulating pathways within intestinal cells, including ISCs and goblet cells, contributing to the maintenance of intestinal barrier function and facilitating repair through the internal non-coding RNAs. Furthermore, exosomes have been found to enhance microcirculation, attenuate in- flammatory responses, and modulate immune responses [42,43]. As a result, HBM-derived exosomes represent a potentially safe, convenient, and effective treatment approach for combating NEC.

3. HBM-derived growth factors

HBM contains various growth factors (GFs) that play a significant role in the development and prevention of necrotizing enterocolitis (NEC) [44]. These GFs have been found to have several beneficial ef- fects, including promoting intestinal cell migration and recovery, enhancing intestinal perfusion, and prolonging the survival of intestinal stem cells [45,46]. Notably, HBM contains GFs such as TGF- , EGF, and IGF [47,48]. TGF- exhibits diverse immunomodulatory activities, including promoting intestinal maturation and defense by converting IgM to IgA in B-lymphocytes, facilitating the production of immune system requirements of infants.

3.1. Epidermal growth factor

Initially discovered in saliva, epidermal growth factor (EGF) main - tains high concentrations in human colostrum, with approximately 50 % of the original content remaining in mature milk from women delivering full-term infants [52–56]. Furthermore, EGF expression is augmented in amniotic fluid, promoting fetal intestinal maturation during the third trimester [57]. EGF plays a crucial role in regulating the growth and maturation of intestinal epithelial cells by binding to its receptors, known as EGF receptors (EGFRs) [58]. Previous research has demon - strated that EGF can stimulate cell proliferation and increase the size and weight of intestinal tissue [59–62]. Fang et al. identified that EGF activates various signaling pathways, including PI3K/AKT, STATS, Ras/MAPK, and PLC- /PKC, through binding to EGFR; thus, regulating the intestinal barrier function [63]. Richard et al. highlighted the significant impact of EGF and TGF- , both trophic peptides in HBM, on the damaged gastrointestinal mucosa [64]. Inhibition of EGF signaling in dams resulted in increased translocation of *E. coli* to splenic tissue, he- patic tissue, and mesenteric lymph nodes in neonatal mice [65]. Addi- tionally, in a rat model, a rat milk substitute (RMS) containing EGF significantly reduced the incidence of NEC by decreasing IL-18 pro- duction [66]. Collectively, the findings from basic and preclinical studies support the potential therapeutic application of EGF in the treatment of NEC.

3.2. Transforming growth factor-beta (TGF-)

TGF- is the predominant cytokine found in HBM, particularly in colostrum. However, its concentration significantly decreases 4–6 weeks after birth [54]. The TGF- family consists of three isoforms: TGF- 1, 2, and 3. TGF- exhibits diverse immunomodulatory activities, including promoting the maturation and defense of the gut by converting IgM to IgA in B-lymphocytes, enhancing the generation of immunoglobulins in the gastrointestinal tract and mammary gland of newborns, and facilitating the recovery of the intestinal mucosa while inducing oral toler - ance [49]. It also exerts anti-inflammatory effects by reducing the levels of pro-inflammatory factors [67]. TGF- plays a crucial role in mucosal repair. In vitro studies utilizing neutralizing antibodies, and in vivo experiments eliminating TGF- from efferocytotic macrophage secre- tome using antibody-coated microbeads have confirmed its effects [68]. Moreover, a feeding trial demonstrated that the addition of TGF- downregulated IL-1 mRNA expression in the mucosa and contributed to 79 % of clinical remission [69]. Furthermore, lower levels of TGF- can worsen the progression of NEC, underscoring its crucial role in the regulation of intestinal tissue [70].

3.3. Insulin-like growth factors

Insulin-like growth factors (IGF-1 and IGF-2) [26] interact with IGFBPs to regulate their bioavailability and stability under normal cir- cumstances [71]. Previous investigations have shown that the concen - tration of IGF-1 is higher in preterm HBM compared to full-term HBM, while there is no significant difference in the concentration of IGF-2 between preterm and full-term HBM [72,73]. The IGFs also exert anti- inflammatory and anti-apoptotic effects by activating the PI3K/AKT pathway [74]. IGF-1 plays a role in promoting the growth of stem cells in intestinal tissue and protecting crypt cells from oxidative injury [75,76]. In a rat model, IGF-1 demonstrated the ability to ameliorate the devel- opment of NEC by inhibiting the inflammatory response [77]. Further - more, in a rat model of hypoxia/reperfusion injury, IGF-1, in combination with erythropoietin, exhibited protective effects against damage and cell apoptosis in the murine intestine [78]. Macrophage- secreted IGF-1 has been shown to enhance endothelial cell growth and mitigate the progression of NEC [79]. Both IGF-1 and IGF-2 promote invasion and tubular formation in human umbilical cord vascular endothelial cells (HUVECs) [80]. Collectively, the available evidence confirms that IGFs can protect against the development of NEC by employing anti-apoptotic and anti-inflammatory mechanisms in models of intestinal injury.

4. HBM-derived oligosaccharides (HMOs)

4.1. The diversity of HMOs

Oligosaccharides are vital components of HBM that significantly affect infant health during breastfeeding. Over 200 HMOs have been identified in HBM, including Lacto-N-neotetraose (LNnT; non- fucosylated) and 2 - fucosyllactose (2 -FL; fucosylated) [13,81,82]. The diversity and concentration of HMOs vary depending on the stage of maternal lactation and the week of infant birth [83]. Oligosaccharides are more abundant in preterm breast milk compared to full-term breast milk. LNnT exhibits high levels of abundance, sialic acid content is elevated during the first month after birth, and fucosylation is not tightly regulated [84].

HMOs are complex carbohydrates that represent the third most abundant substance in HBM [85,86]. Their intricate and unique struc - tures allow them to resist gastrointestinal digestion and hydrolysis by brush-border and pancreatic enzymes, resulting in limited absorption [87]. Preclinical studies have reported several benefits associated with HMOs, including their roles in anti-adhesion, regulation of intestinal epithelial cell responses, modulation, of immunity and the microbiota, prevention of NEC, and enhancement of cerebral development [88–90]. Recent advancements have enabled the isolation and bulk synthesis of HMOs and their analogs. Initial clinical trials conducted on formula-fed infants have shown that supplementing LNnT and 2'-FL is well-tolerated and promotes healthy growth during the first 4–6 months after birth [91,92]. Over the past decade, numerous studies have investigated the impact of HMOs on infant health [93,94]. For instance, Autran et al. examined breast milk samples from a multicenter cohort of 200 mothers, to assess HMO levels and the risk of NEC among breast milk-fed low-birth-weight infants. Their findings demonstrated that high levels of the HMO, disialyllacto-N-tetraose (DSLNT) in breast milk were associated with a reduced risk of NEC among infants (Bell stage 2/3 combined) (OR 0.84; 95 % CI 0.79–0.88; p = 0.001) [95]. However, further studies are needed to explore the factors that influence DSLNT levels in breast milk [96]. In a preclinical study using newborn mice, the addition of HMO-2' FL reduced the severity of NEC, suppressed pro-inflammatory factors in small intestinal tissues, and protected the intestinal structure [97]. Another study found that adding 2'-FL (5 g/L) to the formula had a limited, short-term impact on gut maturation and the risk of NEC in preterm pigs at five days after birth. NEC occurred in 8 out of 16 pigs(50 %) in the 2'-FL group and in 12 out of 17 control pigs (71 %), although the difference in lesion scores was not statistically significant (p = 0.35). Additionally, 2'-FL did not affect specific intestinal parameters [98].

4.2. Effect of HMOs on bacterial residence

Human milk oligosaccharides (HMOs) play a crucial role in shaping the microbiome of breastfed infants [99,100]. They contribute to maintaining a balanced gut microbiome by promoting the growth of beneficial bacteria [94]. Certain Bacteroides species, such as *Bacteroides, fragilis* and *Bacteroides vulgatus*, have been identified as consumers of

HMOs. On the other hand, bacteria like *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *E. coli*, *Clostridium perfringens*, *Eubacterium rectale*, *Veillonella parvula,* and *Enterococcus faecalis* either do not utilize HMOs or do so rarely [101]. The presence of HMOs restricts the avail- ability of nutrients to pathogenic bacteria, inhibiting their growth [102,103]. Various commercial sources of HMOs are available, and HMO-containing formulas have been deemed safe and beneficial for human infants. Therefore, the supplementation of infant formulas with
2'-FL alone or in combination with LNnT has become increasingly common. Furthermore, considering the host defense and immune- enhancing properties of HMOs, they hold potential for immunocom - promised populations and infants susceptible to infections. However, there are a limited number of studies investigating the effects of HMOs
in humans or animals, particularly regarding the administration of HMO mixtures and their impact on immunity. Future research is needed to explore the effects of HMOs on NEC and other relevant aspects.

4.3. Immunomodulatory effect of HMOs

Human milk oligosaccharides (HMOs) exhibit immunomodulatory effects in infants,. although the specific effects may vary among milk donors due to differences in HMO components and levels [13,104]. These effects are associated with enhancing immunity, influencing the intestinal microbiota, and maintaining the integrity of the intestinal epithelial barrier, which may contribute to protection against inflam - mation and NEC [60]. HMOs display a high degree of diversity and regulate both adaptive and innate immunity in newborns [105]. Certain
HMO structures, such as 2'-FL, have been found to suppress Toll-like receptor-4 (TLR4) activation, illustrating a structure-specific effect [106]. Additionally, HMOs with lower abundance, like DSLNT have demonstrated the ability to reduce the incidence of NEC in experimental studies [107]. In in vitro experiments and animal models, HMOs directly interact with immune cells in mucosal and systemic compartments, as well as with gastrointestinal epithelial cells, to modulate immune re- sponses [108].

HMOs serve as a protective barrier against pathogens by competi - tively binding to them, preventing their attachment to or invasion of the intestinal epithelium [93]. Furthermore, HMOs can directly modulate the responses of immune cells to pathogens, such as 2'-FL, which sup- presses inflammation triggered by lipopolysaccharides (LPS) during
E. coli invasion of IECs [104]. Additionally, HMOs exert various pro- tective effects on IECs, including anti-apoptotic, anti-inflammatory and anti- oxidative effects [109].

5. Conclusions and perspectives

NEC is a prevalent and severe gastrointestinal disease affecting ne- onates, particularly premature infants, and poses a significant threat to their survival [1]. Currently, there are no specific treatment modalities for NEC, and management primarily focuses on symptomatic measures such as fasting, gastrointestinal decompression, intravenous nutrition support and anti-infection therapy. Surgical intervention is considered when conservative approaches fail, but it often leads to long-term complications such as short bowel syndrome, intestinal stenosis, and neurodevelopmental disorders, imposing substantial burdens on fam- ilies and society [110,111]. Consequently, there is an urgent need to comprehensively understand the pathogenesis of NEC and develop effective preventive and therapeutic strategies.

Numerous studies have highlighted the protective effects of HBM against NEC [112–114]. The beneficial properties of HBM can be attributed to its biologically active constituents, which influence intes-
tinal growth, barrier function, microvascular development, and immu - nological maturation. Preterm infants who are fed HBM have a lower incidence of NEC compared to those fed infant formula [115,116]. HBM provides immunological, nutritional, and developmental advantages to
the growing newborn through various molecular and cellular mechanisms.

To optimize therapeutic outcomes, it is crucial to unravel the mo- lecular pathways underlying the beneficial effects of HBM in alleviating
NEC (Fig. 1). HBM-derived exosomes, GFs and oligosaccharides have demonstrated significant benefits in preclinical and experimental studies and hold promise as potential treatments for NEC. Further in- vestigations are warranted to elucidate the underlying mechanisms and advance the bioengineering, preparation, and applications of HBM- derived exosomes, GFs, and oligosaccharides. Rigorous clinical trials are also needed to establish the efficacy of these interventions as novel therapeutic options for NEC.

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CRediT authorship contribution statement

CCF, XYW and XPZ were involved in the conception and design of the study. CCF drafted the manuscript, and WQS participated in the revision of the manuscript. XYW and XPZ reviewed the manuscript. All authors read and approved the final draft.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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