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Cytoprotective effects of galacto-oligosaccharides on colon epithelial cells via up-regulating miR-19b

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ABSTRACT

Aims: Despite the protective effect of galacto-oligosaccharides (GOS) on human colon has been widely-reported, the mechanism of its beneficial effect is still unclear. This paper aims to reveal the internal mechanism underlined the anti-colitis effect of GOS by studying its regulatory effect on miRNAs.

Main methods: An in vitro model of colitis was constructed by using human colon epithelial FHC cells and lipopolysaccharide (LPS). An in vivo colitis model was established as well, by injecting $Rag2^{-/-}$ Sprague-Dawley (SD) rats with helicobacter hepaticus. The effects of GOS pre-treatment on these two models were tested, and the miRNAs involved in these effects were studied.

Key findings: The expression of miR-19b, miR-590-5p and miR-495 was up-regulated, and the expression of miR-29a, miR-31 and miR-142-5p was down-regulated by GOS treatment in both normal and LPS-stimulated FHC cells. Among which, miR-19b was the most varied miRNA. GOS pre-treatment significantly attenuated LPS-induced cell injury, as evidenced by the increase of cell viability, the decrease of apoptosis, as well as the suppressed release of TNF-α, IFN-γ and IL-1β. GOS pre-treatment could also prevent $Rag2^{-/-}$ rats against helicobacter hepaticus injection induced diarrhea and inflammation, as the body weight and colon organ weight were recovered, diarrhea score was declined, and the release of pro-inflammatory cytokines was inhibited. The in vitro and in vivo effects of GOS abovementioned were all impeded when miR-19b was silenced.

Significance: In vitro and in vivo experiments showed that GOS have certain anti-colitis effect, and this effect may be achieved by up-regulating miR-19b.

1. Introduction

Colitis is an inflammatory disease of the colon which can be caused by injection of bacteria, fungi, viruses, parasites, protozoa and other organisms, as well as several allergic reactions, like physical and chemical factors. The main clinical manifestations are diarrhea, abdominal pain and constipation [1,2]. Depending on the causes of colitis, many types of colitis are classified. Among which, Crohn's disease (CD) and Ulcerative colitis (UC) are identified as two main types caused by autoimmunity. And thereby, the link between bacteria injection and CD/UC has been well-established [3,4]. Inhibition of inflammation caused by bacterial infection can effectively relieve CD and UC. Besides, the complex symbiotic system of aerobic and anaerobic bacteria provides stable antigen-driven in chronic immune-mediated colitis [5].

Galacto-oligosaccharides (GOS) are functional oligosaccharides with natural properties. GOS are a mixture of substances produced by lactose. The mixture consists of 2 to 8 sugar units with glucose at the end and the remaining sugar units are galactose and disaccharide consisting of two molecules of galactose. Recently, GOS have been widely-considered as beneficial nutrients in human gastrointestinal tract. They are capable of altering the composition and metabolism of the gut microbiota, and thus offering beneficial effects in human health [6]. For instance, GOS can promote the growth of *Lactobacillus*, *Streptococcus* and *Bifidobacterium* [7]. Apart from the potential of GOS in modifying the gut microbial balance, GOS were found to inhibit injections by many pathogens due to their ability in coating the surface of gastrointestinal epithelial cells [8,9]. However, the mechanism underlined the colon-protecting effects of GOS is not studied in exhaustive details.

MicroRNAs (miRNAs) are a class of endogenous non-coding RNA with regulatory functions found in eukaryotes, with a size of about 20–25 nucleotides [10,11]. Recent studies have shown that miRNA is

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involved in a variety of regulatory pathways, including nutritional control, virus defense, hematopoietic process, organ formation, cell proliferation and apoptosis, fat metabolism, etc. [12–18]. Aberrant expression of miRNAs is often occurred in patients with colitis, and these miRNAs have been considered as potential biomarkers for the diagnosis of this disease [19]. By retrieving though the published reports, miR-148a, miR-206, miR-19b, miR-29a, miR-590-5p, miR-146a-5p, miR-495, miR-106a, miR-155, miR-31 and miR-142-5p are shown to play promoting or inhibitory effects on the initiation and development of colitis [20–26].

Mammalian intestine is an important organ, in where nutrients are absorbed and immune response is happened. Besides, miRNAs are appeared to play critical roles in both the crypt-villus axis of cellular self-renewal and inflammation in the mammalian intestinal mucosa and their impact on the microbiota [27]. Thus, we are interested in investigating whether GOS exert its anti-colitis effects via regulating colitis-associated miRNAs. This paper aims to reveal the internal mechanism underlined the anti-colitis effect of GOS by studying its regulatory effect on miRNAs.

2. Materials and methods

2.1. Human colon epithelial cells culture and treatment

Human colon epithelial cell line FHC (Catalogue number: ATCC® CRL-1831™) was purchased from ATCC (Manassas, VA). The cultivating methods can be found in ATCC's official website. In brief, FHC cells were cultured in DMEM:F12 medium (ATCC) supplied with 10 mM HEPES, 0.005 mg/mL insulin, 0.005 mg/mL transferrin, 100 ng/mL hydrocortisone, 20 ng/mL human recombinant EGF (all from Sigma, St. Louis, MO), 25 ng/mL cholera toxin (Invitrogen, Carlsbad, CA), and 10% fetal bovine serum (Hyclone, Logan, UT). Cells were routinely grown in 75 cm^2 flask at 37 °C in a humid atmosphere with 5% CO_2.

Lipopolysaccharide (LPS) from *Escherichia coli* O111:B4 was purchased from Sigma. FHC cells were treated with 1 μg/mL of LPS for 24 h [28] to induce in vitro inflammation.

Guaranteed reagent grade of GOS with purity > 99.8% was purchased from Grbio Biotechnology Co., Ltd. (Shanghai, China). GOS with a final concentration of 2% was used to treat FHC cells before LPS stimulation for 24 h [29].

2.2. miRNA transfection

miR-19b inhibitor and its negative control (NC) were purchased from GenePharma (Shanghai, China). The sequences of miR-19b used were as follows: UAACCGAUUUCAGAUGGUGCUA. miR-19b inhibitor and NC with final concentration of 100 nM were respectively transfected into FHC cells with the mediation of Lipofactamine 2000 reagent (Invitrogen). After 48 h of transfection, the efficiency of the transfection was tested by qRT-PCR analysis.

2.3. qRT-PCR analysis

For detection of miRNAs expression, total miRNAs were extracted from FHC cells by using RNAiso for Small RNA (Takara, Dalian, China). Reverse transfection and qRT-PCR processes were carried out with the mediation of Mir-X™ miRNA First-Strand Synthesis Kit and Mir-X™ miRNA qRT-PCR TB Green™ Kit (both from Takara), respectively. For the test of mRNA expression, cellular mRNAs were isolated by using Trizol reagent (Invitrogen). PrimeScript™ RT Master Mix and TB Green™ Premix Ex Taq™ II obtained from Takara were used for cDNA synthesis and qRT-PCR. The amounts of the targeted miRNAs and mRNAs were standardized against U6 snRNA and GAPDH, respectively. Data were calculated according to $2^{-\Delta\Delta Ct}$ method [30].

2.4. Detection of cell viability

FHC cells (1×10^3) in 96-well plates were treated as indicated. After which, cell viability was measured at 48 h using cell counting kit-8 (Dojindo Laboratories, Kumamoto, Japan), according to the manufacturer's instructions. Optical density (OD) values of each well were recorded by an ELISA reader (Bio-Rad Laboratories, Hercules, CA) for calculating relative cell viability.

2.5. Detection of apoptosis

After the indicated treatment, 1×10^5 FHC cells per sample were collected and washed twice with PBS. The cell apoptosis was detected by the Annexin V-FITC/PI apoptosis detection kit (Beyotime, Shanghai, China), according to the manufacturer's instructions. The stained cells were evaluated by flow cytometric analysis. Annexin V-positive and PI-negative cells are considered as apoptotic cells and the rate of apoptotic cells was calculated by using the FlowJo software (TreeStar, San Carlos, CA).

2.6. Western blotting

The total protein in FHC cells after the indicated treatment was extracted by using RIP lysis buffer (Beyotime). Protein concentration of the extracts was measured by BCA Protein Assay Kit (Sangon Biotech, Shanghai, China). Proteins were separated by SDS-PAGE and transferred onto PVDF membranes. For immunoblotting, the following primary antibodies were used: anti-Bax (ab32503), anti-Bcl-2 (ab182858), anti-cleaved caspase-3 (ab2302), and anti-GAPDH (ab181603). Goat anti-rabbit IgG H&L (HRP) (ab7090) was used as a secondary antibody. Electrochemiluminescence Western blotting kit (Thermo Fisher Scientific, Waltham, MA) was used for developing the target bands.

2.7. Animals and in vivo study design

A total of 40 SPF grade of Sprague-Dawley (SD) rats ($Rag2^{-/-}$) (20 males and 20 females, 6 weeks old) were purchased from Vital River Laboratories (Beijing, China) and cultured under SPF conditions as described elsewhere [31]. The $Rag2^{-/-}$ rats are high immunodeficient with dysfunction of B and T cells. All animal experiments were approved by the Animal Ethics Committee of Northeast Agricutural University.

The rats were randomly divided into 5 groups (8 rats per group): Control, HH, HH + GOS, HH + GOS + NC and HH + GOS + miR-19b inhibitor. Rats in the Control group received no treatment. HH group rats were infected with 0.3 mL helicobacter hepaticus suspension (OD values ≥ 1.8 at 600 nm) via oral for 2 consecutive days. The number of dosing of helicobacter hepaticus suspension to rats was two times per day (at morning and night). Bacterial culture was performed as previously described [32]. In the HH + GOS group, the rats were continuously administrated with GOS via oral for 2 weeks at a dose of 5000 mg/day/kg before helicobacter hepaticus infection. GOS was dissolved in double-distilled H_2O and given to rats two times per day (at morning and night). Rats in the HH + GOS + NC and HH + GOS + miR-19b inhibitor groups received 60 μg/kg of NC or miR-19b inhibitor injection (intraperitoneal) before GOS treatment and helicobacter hepaticus infection.

The severity of diarrhea was evaluated according to the following criterion which is scored from 1 to 4: no diarrhea (1), fecal specimens loose yellow-green indicates mild diarrhea (2), fecal specimens totally loose yellow-green indicates moderate diarrhea (3), fecal specimens with high water content indicates severe diarrhea (4) [33]. The rats with scores ≥ 2 were considered for use as animal model of colitis. After the end of the experiment, diarrhea index was detected every 4 days. Also, the weight of body and colon organ was weighed.

2.8. ELISA

Concentrations of TNF-α, IFN-γ, IL-1β in rat serum and cell culture supernatant were measured using the ELISA kit (Catalogue number: ab46070, ab46107 and ab100768, Abcam), according to the manufacturer's instructions.

2.9. Statistics

All data presented as mean ± SD. In vivo experiments were repeated in eight rats, while in vivo experiments were done in triplicate. SPSS software version 19.0 (Chicago, IL) was sued for statistical analysis. Statistical difference between groups was analyzed by one way ANOVA followed by Duncan post-hoc. Results were considered statistically significant at $P < 0.05$.

3. Results

3.1. Six miRNAs could be altered by GOS treatment

Through literature review, 11 miRNAs closely related to colitis were screened to study whether GOS could regulate the expression of these miRNAs [34–37]. The results of Fig. 1A showed that GOS up-regulated the expression of miR-19b, miR-590-5p and miR-495, and down-regulated the expression of miR-29a, miR-31 and miR-142-5p in normal FHC cells ($P < 0.05$). However, the expression levels of miR-148a, miR-206, miR-146a-5p, miR-106a and miR-155 could not be significantly altered by GOS ($P > 0.05$).

The same trend was obtained in LPS-damaged FHC cells. Data in Fig. 1B showed that, the expression of miR-19b, miR-590-5p, miR-495, miR-29a, miR-31 and miR-142-5p in LPS-injured FHC cells was significantly different from that in LPS-injured FHC cells treated with GOS ($P < 0.05$).

Either in normal or LPS-injured FHC cells, miR-19b is the most varied miRNA by GOS, thus we chose miR-19b for further study.

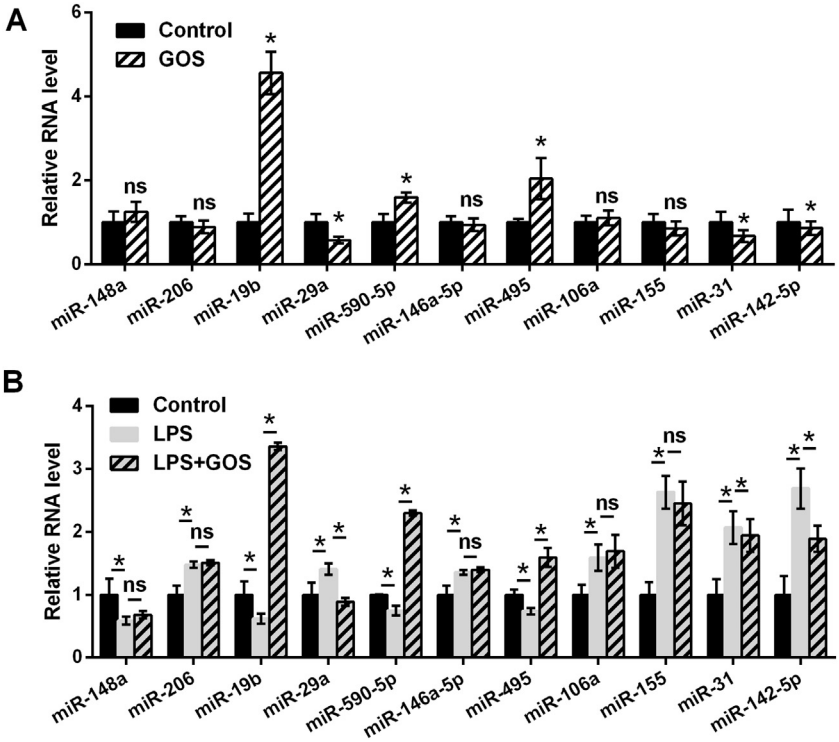

Fig. 1. Six miRNAs are altered by GOS. (A) The expression of 11 miRNAs closely related to colitis in FHC cells after treating with GOS. (B) The expression of these 11 miRNAs in FHC cells after LPS stimulation, and LPS plus GOS treatment. qRT-PCR analysis was performed for testing miRNA expression. Data are expressed as mean ± SD ($n = 3$). ns, no significance; *, $P < 0.05$.

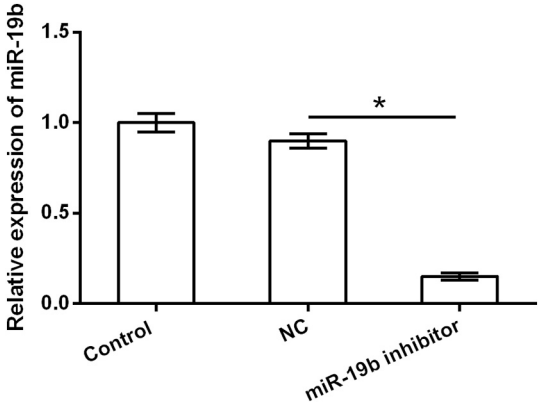

Fig. 2. Silence of miR-19b by transfection. Relative expression of miR-19b in FHC cells, after transfection with miR-19b inhibitor or its negative control (NC). qRT-PCR analysis was performed for testing miR-19b expression. Data are expressed as mean ± SD ($n = 3$). *, $P < 0.05$.

3.2. The expression of miR-19b was silenced by transfection

To investigate whether miR-19b is a downstream gene which plays an anti-colitis role in cell treated with GOS, the expression of miR-19b in cell was silenced by inhibitor transfection. The qRT-PCR data showed that the expression of miR-19b in the miR-19b inhibitor group was significantly lower than that in the NC group ($P < 0.05$, Fig. 2), indicating that miR-19b silenced cells were successfully obtained.

3.3. GOS protected FHC cells against LPS induced injury via up-regulating miR-19b

Next, FHC cells were transfected with miR-19b inhibitor and then treated with GOS and LPS, to see the involvement of miR-19b expression in GOS's function. As shown in Fig. 3A–C, LPS significantly reduced cell viability while induced apoptosis of FHC cells ($P < 0.05$). Pretreatment with GOS attenuated LPS induced injury to FHC cells, as cell

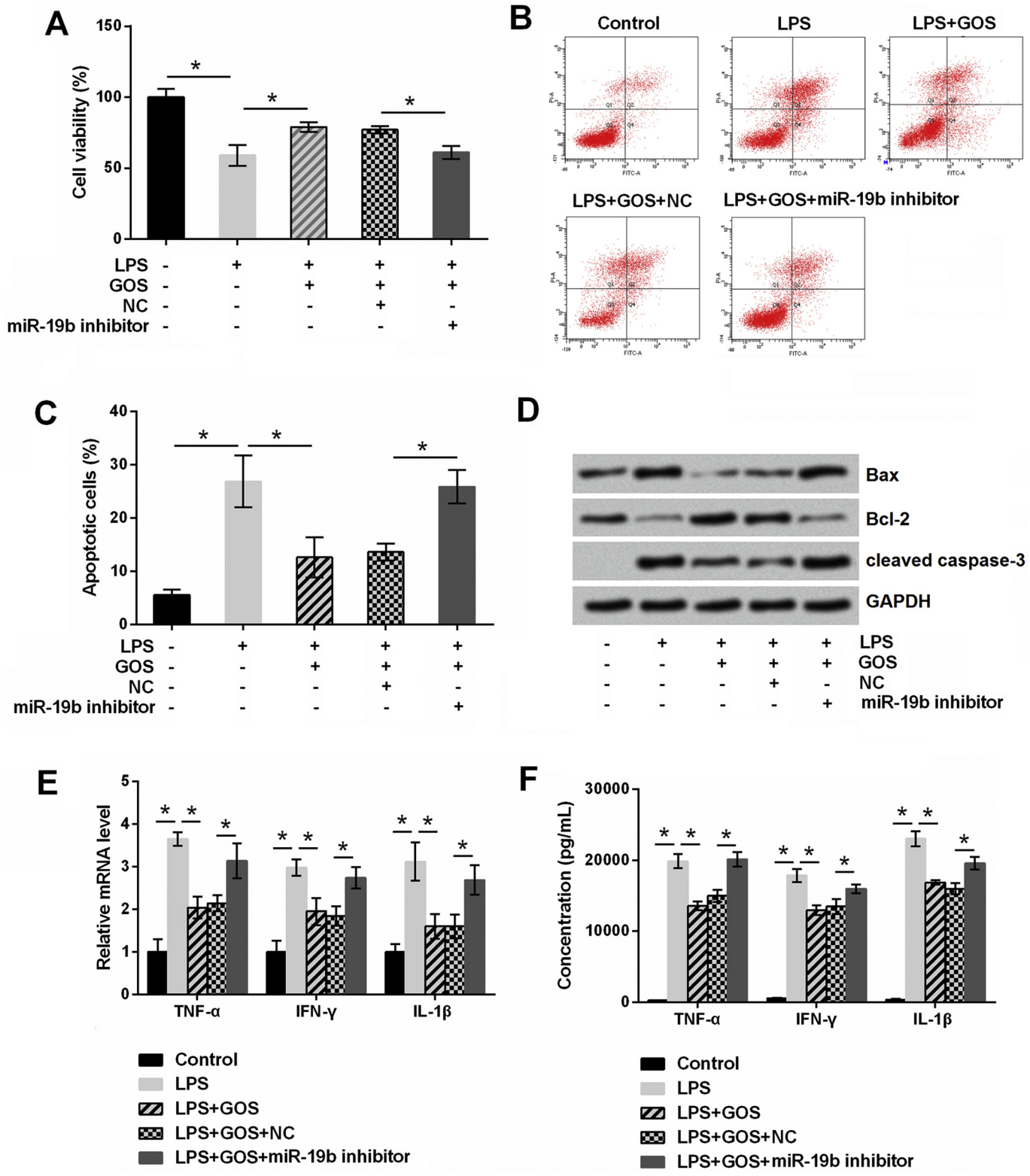

Fig. 3. In vitro effects of GOS on LPS-injured FHC cells via regulating miR-19b. FHC cells were transfected with miR-19b inhibitor or its negative control (NC), after which the cells were treated by LPS or LPS plus GOS. (A) Cell viability was detected by CCK-8 assay. (B—C) Cells were stained with Annexin V-FITC and PI, and the apoptosis cells were analyzed by flow cytometry. (D) The protein levels of Bax, Bcl-2 and cleaved caspase-3 were tested by western blot analysis. (E) The relative mRNA levels of TNF-α, IFN-γ and IL-1β by qRT-PCR. (F) The concentrations of TNF-α, IFN-γ, IL-1β by ELISA. Data are expressed as mean ± SD ($n = 3$). *, $P < 0.05$.

viability was enhanced, and apoptosis was suppressed ($P < 0.05$). More interestingly, GOS pre-treatment could not protect FHC cells against LPS-induced cell death when miR-19b was silenced by inhibitor transfection ($P < 0.05$). Results from western blot further confirmed this observation, as Bax and cleaved caspase-3 was remarkably down-regulated, while Bcl-2 was up-regulated following GOS treatment (Fig. 3D). And next, these alterations induced by GOS were abolished by miR-19b silence.

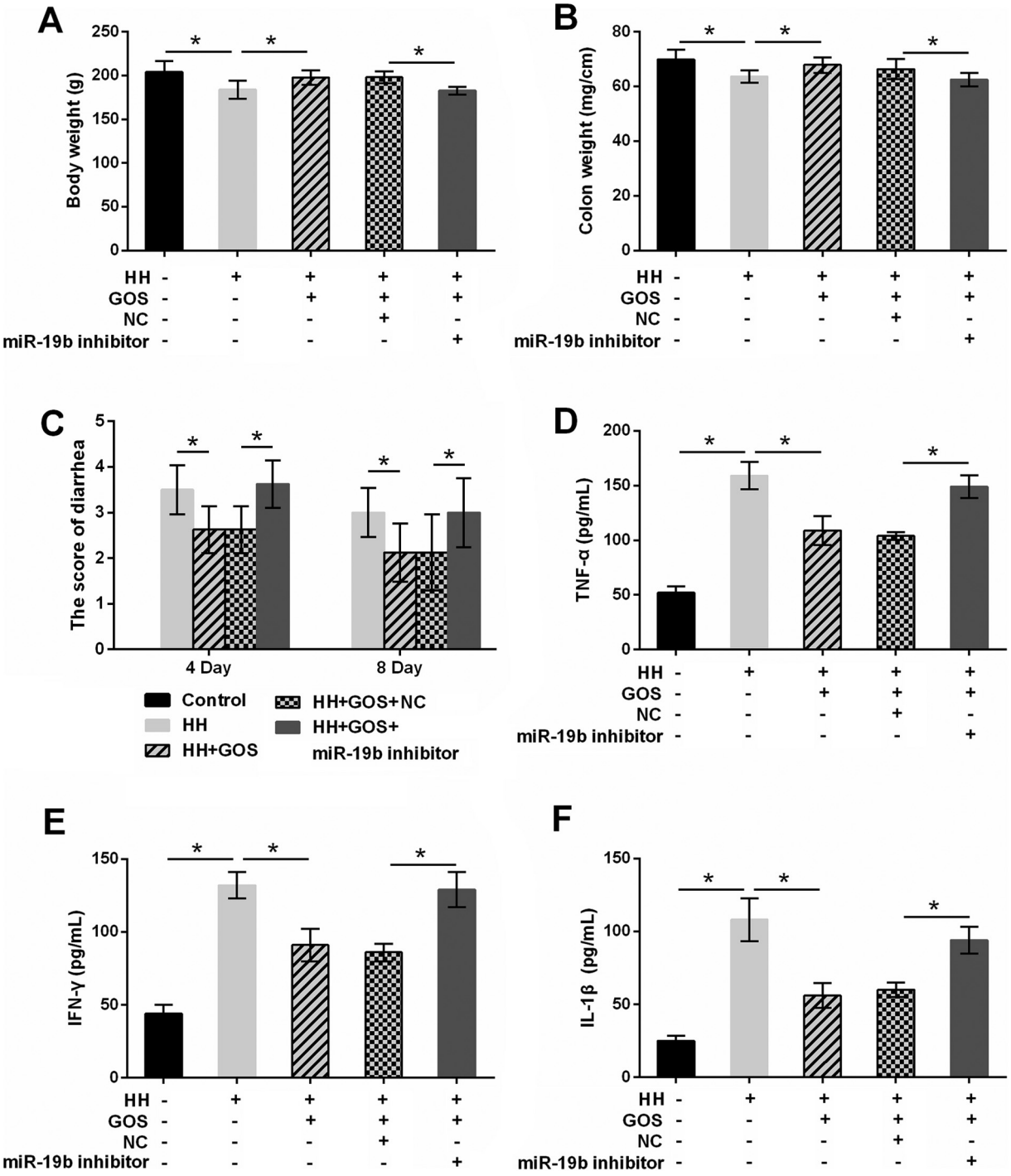

Fig. 4. In vivo effects of GOS on Rag2$^{-/-}$ SD rats injected with helicobacter hepaticus via regulating miR-19b. Rag2$^{-/-}$ SD rats were transfected with miR-19b inhibitor or its negative control, after which received helicobacter hepaticus injection or in combination with GOS pre-treatment. Changes of (A) body weight, (B) colon organ weight, and (C) diarrhea index of rats were recorded. The concentrations of (D) TNF-α, (E) IFN-γ, and (F) IL-1β in rat serum were measured by ELISA. Data are expressed as mean ± SD ($n = 8$). *, $P < 0.05$.

Same trends were observed in the release of pro-inflammatory cytokines. From Fig. 3E and F, we found that the mRNA levels and the protein concentrations of TNF-α, IFN-γ and IL-1β were all increased by LPS ($P < 0.05$). GOS pre-treatment attenuated LPS-stimulated TNF-α, IFN-γ and IL-1β ($P < 0.05$), while the impacts of GOS on these pro-inflammatory cytokines were impeded when miR-19b was silenced ($P < 0.05$). All these results suggested that GOS protected FHC cells against LPS induced injury at least in part via up-regulating miR-19b.

GOS attenuated helicobacter hepaticus induced colitis via up-regulating miR-19b.

As the cytoprotective effects of GOS have been identified, we further verified the protective effects of GOS in an animal model of colitis induced by helicobacter hepaticus. Fig. 4A and B showed that, the weight of whole body and colon organ of Rag2$^{-/-}$ SD rats were significantly reduced after helicobacter hepaticus injection as compared with control group ($P < 0.05$). Treatment with GOS beforehand obviously recovered the weight loss caused by helicobacter hepaticus ($P < 0.05$). However, GOS did not alleviate the weight loss caused by helicobacter

hepaticus when miR-19b was silenced ($P < 0.05$). In Fig. 4C, the result trend is similar to that of Fig. 4A and B, which reflects diarrhea index. Specifically, rats still had an attack of diarrhea at the 4th day after helicobacter hepaticus injection, and the diarrhea was slightly relieved with time increasing. The rats received GOS pre-treatment processed low diarrhea scores as compared to the rats injected with helicobacter hepaticus alone ($P < 0.05$). Moreover, GOS pre-treatment could not attenuate diarrhea such significant when miR-19b was silenced by gene transfer ($P < 0.05$).

Fig. 4D–F showed that the concentrations of TNF-α, IFN-γ and IL-1β in rat serum were obviously elevated by helicobacter hepaticus injection ($P < 0.05$). Treatment with GOS beforehand significantly reduced the concentrations of pro-inflammatory cytokines caused by helicobacter hepaticus ($P < 0.05$). Not surprisingly, GOS could not reduce the concentrations of these pro-inflammatory cytokines when miR-19b was silenced. Thereby, it is rationally to draw a conclusion that GOS attenuated helicobacter hepaticus induced colitis at least in part via up-regulating miR-19b.

4. Discussion

It is well-known that nutrients play significantly roles in maintaining human health and the management of many diseases. Recent decades, a growing number of reports focused on investigating the beneficial effects of various nutrients and the mechanisms involved. GOS are functional ingredients produced by lactose. The beneficial effects of GOS have long been known, and three mechanisms of theirs action have been roughly revealed. One is related to the growth promoting effects of GOS on several probiotics, like *Lactobacillus*, *Streptococcus* and *Bifidobacterium* [7]. One is related to the production of short chain fatty acids, which is helpful for inhibiting the growth of undesirable microorganisms [38]. And other one is related to the inhibitory effects on pathogen injections, due to the ability of GOS in coating the surface of gastrointestinal epithelial cells [8,9]. However, a recent paper found that GOS were able to increase sucrase activity in cultured human epithelial intestinal cells, and thus the authors hypothesized that GOS may act directly on these cells [39]. A later study further confirmed this hypothesis, that GOS could directly protect human epithelial intestinal cells against deoxynivalenol induced epithelial barrier dysfunction, and inhibited the release of IL-8 from cell [29]. The abovementioned three mechanisms cannot explain how GOS impact human epithelial intestinal cells directly. Therefore, we are interested in this point and found that GOS exerted theirs beneficial effects on the human colon epithelial cells may partially via up-regulating miR-19b. This finding allows us to understand the beneficial effects of GOS in a different mechanism.

In the current study, in vitro experiments were performed and the cytoprotective effects of GOS on human colon epithelial cells (FHC) were observed. Pre-treatment with GOS attenuated LPS-inhibited cell viability and LPS-induced apoptosis. This phenomenon was coupled with the down-regulated Bax, the up-regulated Bcl-2 and the suppressed cleavage of caspase-3. These three proteins are all key control points for apoptosis pathways. Bax gene, belongs to the Bcl-2 gene family, is the most important apoptotic gene in human body. The encoded Bax protein can form a heterodimer with Bcl-2 and has an inhibitory effect on Bcl-2. Caspase-3 is one of the main executors of apoptosis. Thus, our data suggested that pre-treating FHC cells with GOS significantly attenuated LPS-induced cell death. Moreover, GOS were found to prevent LPS induced the release of pro-inflammatory cytokines, including TNF-α, IFN-γ and IL-1β, indicating GOS have potent anti-inflammatory effects. This finding was in line with multiple previous studies. For instance, Gopalakrishnan et al. found that GOS have the potential to regulate colon inflammation and thus reduce the severity of colitis by initiating non-specific immunity [32]. Bouwhuis et al. demonstrated that pigs supplemented with GOS resulted in reduced mRNA levels of IL-6, IL-22 and TNF-α [40].

The colon-protecting effects of GOS were also confirmed in an animal model in this study. By injection with helicobacter hepaticus for two consecutive days, $Rag2^{-/-}$ SD rats had a severe attack of diarrhea. The body weight and colon organ weight begun to loss, and the levels of IL-6, IL-22 and TNF-α in rat serum were dramatically increased. These observations indicated that $Rag2^{-/-}$ SD rats suffered from colitis after helicobacter hepaticus injection. Similar murine models of colitis were used elsewhere. For instance, Smad3- and IL10-deficient mice injection with helicobacter hepaticus leads to a severe colitis [32,41]. These animal models were established due to the immune system was suppressed which made animals more sensitive to bacterial infection [42,43]. Besides this, other animal models of colitis were also utilized in recent studies, like dextran sulfate sodium (DSS)-induced colitis model. DSS is a long chain polymer of sulfated glucose that can induce epithelial damage. DSS is the most widely used stimulation for the establishment of colitis animal model, as its predominant properties, like rapidity, simplicity, reproducibility and controllability [44]. Despite that, we used immunodeficiency rat and helicobacter hepaticus injection for the establishment of colitis model. And found that treatment with GOS beforehand could effectively relieve the severity of illness. These finding indicates that, GOS may have potentials in preventing colitis among immunodeficiency population.

With the better understanding of miRNAs, the importance of them in the onset and progression of colitis has been highlighted. Some of them have been considered as potential biomarkers for this disease, as they are differentially expressed in patients with colitis. For example, Wu et al. found out that the expression of 11 kinds of miRNAs in the intestinal mucosa was different during the activity period of UC patients, of which 3 kinds decreased significantly (miR-192, miR-375 and miR-422b) and 8 kinds increased significantly (miR-16, miR-21, miR-23a, miR-24, miR-19b, miR-126, miR-195 and Let-7f) [34]. Besides, the functional roles and potential usage as therapeutic targets were widely reported [45], since they play important roles in regulating cell proliferation, apoptosis, differentiation and inflammatory responses. Moreover, host miRNAs in mammalian cells appeared to work as crucial regulators during the injection of diverse pathogens. They participate in regulating the self-renewal and inflammation of intestinal mucosal epithelia [27], in where GOS absorption and bacterial infection were carried out. Bases on these reasons, we are interested in studying whether GOS protected FHC cells and SD rats via modulating miRNAs.

By retrieving though the published reports, 11 kinds of colitis-associated miRNAs [20–26] were selected. We found that 6 of them can be altered by GOS treatment. The expression of miR-19b, miR-590-5p and miR-495 was up-regulated, and the expression of miR-29a, miR-31 and miR-142-5p was down-regulated by GOS. Among them, GOS had the most significant effect on miR-19b expression, thus miR-19b was selected for further investigation. The present work showed that the in vitro and in vivo colon-protective effects of GOS were both attenuated when miR-19b was silenced. This finding suggested that GOS could prevent colitis to a certain degree via partially up-regulating miR-19b. Actually, the functional effects of miR-19b have been reported previously. Chen et al. found that inhibition of miR-19b elevated the expression of TNF-α, IL-8, and GM-GSF, suggesting the anti-inflammatory effects of miR-19b [46]. Zhou et al. revealed that miR-19b could target TNF-α and thereby suppressed the progression of UC [47]. Further studies are required to investigate the miRNAs other than miR-19a which can act as downstream effectors of GOS.

5. Conclusions

In conclusion, in vivo and in vitro experiments in the current study showed that GOS have certain anti-colitis effect, and this effect may be achieved by regulating miR-19b. The finding of this study allows a closer understanding of the different mechanism involved in the beneficial effects of GOS.

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Authorship

Conceives, designed the experiments and wrote the paper: Guofang Zhang and Jinwei Sun. Performed the experiments and analyzed the data: Jinwei Sun, Wenxing Liang, Xiaofeng Yang and Qiming Li.

Declaration of Competing Interest

Authors declare that there is no conflict of interests.

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References

[1] M.F. Neurath, Animal models of inflammatory bowel diseases: illuminating the pathogenesis of colitis, ileitis and cancer, Dig. Dis. 30 (Suppl. 1) (2012) 91–94.
[2] C. Kunz, S. Rudloff, W. Baier, N. Klein, S. Strobel, Oligosaccharides in human milk: structural, functional, and metabolic aspects, Annu. Rev. Nutr. 20 (2000) 699–722.
[3] R.J. Xavier, D.K. Podolsky, Unravelling the pathogenesis of inflammatory bowel disease, Nature 448 (2007) 427–434.
[4] R.B. Sartor, Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics, Gastroenterology 126 (2004) 1620–1633.
[5] H.C. Rath, M. Schultz, R. Freitag, L.A. Dieleman, F. Li, H.J. Linde, J. Scholmerich, R.B. Sartor, Different subsets of enteric bacteria induce and perpetuate experimental colitis in rats and mice, Infect. Immun. 69 (2001) 2277–2285.
[6] S. Macfarlane, G.T. Macfarlane, J.H. Cummings, Review article: prebiotics in the gastrointestinal tract, Aliment. Pharmacol. Ther. 24 (2006) 701–714.
[7] A. Cardelle-Cobas, N. Corzo, A. Olano, C. Pelaez, T. Requena, M. Avila, Galactooligosaccharides derived from lactose and lactulose: influence of structure on Lactobacillus, Streptococcus and Bifidobacterium growth, Int. J. Food Microbiol. 149 (2011) 81–87.
[8] K. Shoaf, G.L. Mulvey, G.D. Armstrong, R.W. Hutkins, Prebiotic galactooligosaccharides reduce adherence of enteropathogenic Escherichia coli to tissue culture cells, Infect. Immun. 74 (2006) 6920–6928.
[9] H. Kittana, M.I. Quintero-Villegas, L.B. Bindels, J.C. Gomes-Neto, R.J. Schmaltz, R.R. Segura Munoz, L.A. Cody, R.A. Moxley, J. Hostetter, R.W. Hutkins, A.E. Ramer-Tait, Galactooligosaccharide supplementation provides protection against Citrobacter rodentium-induced colitis without limiting pathogen burden, Microbiology 164 (2018) 154–162.
[10] D.P. Bartel, MicroRNAs: genomics, biogenesis, mechanism, and function, Cell 116 (2004) 281–297.
[11] I. Alvarez-Garcia, E.A. Miska, MicroRNA functions in animal development and human disease, Development 132 (2005) 4653–4662.
[12] S. Lin, R.I. Gregory, MicroRNA biogenesis pathways in cancer, Nat. Rev. Cancer 15 (2015) 321–333.
[13] L.J. Simpson, K.M. Ansel, MicroRNA regulation of lymphocyte tolerance and autoimmunity, J. Clin. Invest. 125 (2015) 2242–2249.
[14] E.N. Nolte-'t Hoen, E. Van Rooij, M. Bushell, C.Y. Zhang, R.H. Dashwood, W.P. James, C. Harris, D. Baltimore, The role of microRNA in nutritional control, J. Intern. Med. 278 (2015) 99–109.
[15] S. Chandra, D. Vimal, D. Sharma, V. Rai, S.C. Gupta, D.K. Chowdhuri, Role of miRNAs in development and disease: lessons learnt from small organisms, Life Sci. 185 (2017) 8–14.
[16] S.H. Hong, K.S. Kim, I.H. Oh, Concise review: exploring miRNAs–toward a better understanding of hematopoiesis, Stem Cells 33 (2015) 1–7.
[17] S. Riccardi, S. Bergling, F. Sigoillot, M. Beibel, A. Werner, J. Leighton-Davies, J. Knehr, T. Bouwmeester, C.N. Parker, G. Roma, B. Kinzel, MiR-210 promotes sensory hair cell formation in the organ of corti, BMC Genomics 17 (2016) 309.
[18] M. Rebucci, A. Sermeus, E. Leonard, E. Delaive, M. Dieu, M. Fransolet, T. Arnould, C. Michiels, miRNA-196b inhibits cell proliferation and induces apoptosis in HepG2 cells by targeting IGF2BP1, Mol. Cancer 14 (2015) 79.
[19] C.G. Chapman, J. Pekow, The emerging role of miRNAs in inflammatory bowel disease: a review, Ther. Adv. Gastroenterol. 8 (2015) 4–22.
[20] F. Wu, M. Zikusoka, A. Trindade, T. Dassopoulos, M.L. Harris, T.M. Bayless, S.R. Brant, S. Chakravarti, J.H. Kwon, MicroRNAs are differentially expressed in ulcerative colitis and alter expression of macrophage inflammatory peptide-2 alpha, Gastroenterology 135 (2008) 1624–1635.e24.
[21] T. Takagi, Y. Naito, K. Mizushima, I. Hirata, N. Yagi, N. Tomatsuri, T. Ando, Y. Oyamada, Y. Isozaki, H. Hongo, K. Uchiyama, O. Handa, S. Kokura, H. Ichikawa, T. Yoshikawa, Increased expression of microRNA in the inflamed colonic mucosa of patients with active ulcerative colitis, J. Gastroenterol. Hepatol. 25 (Suppl. 1) (2010) S129–S133.
[22] M. Fasseu, X. Treton, C. Guichard, E. Pedruzzi, D. Cazals-Hatem, C. Richard, T. Aparicio, F. Daniel, J.C. Soule, R. Moreau, Y. Bouhnik, M. Laburthe, A. Groyer, E. Ogier-Denis, Identification of restricted subsets of mature microRNA abnormally expressed in inactive colonic mucosa of patients with inflammatory bowel disease, PLoS One 5 (2010).
[23] Z. Bian, L. Li, J. Cui, H. Zhang, Y. Liu, C.Y. Zhang, K. Zen, Role of miR-150-targeting c-Myb in colonic epithelial disruption during dextran sulphate sodium-induced murine experimental colitis and human ulcerative colitis, J. Pathol. 225 (2011) 544–553.
[24] J.R. Pekow, U. Dougherty, R. Mustafi, H. Zhu, M. Kocherginsky, D.T. Rubin, S.B. Hanauer, J. Hart, E.B. Chang, A. Fichera, L.J. Joseph, M. Bissonnette, miR-143 and miR-145 are downregulated in ulcerative colitis: putative regulators of inflammation and protooncogenes, Inflamm. Bowel Dis. 18 (2012) 94–100.
[25] M. Coskun, J.T. Bjerrum, J.B. Seidelin, J.T. Troelsen, J. Olsen, O.H. Nielsen, miR-20b, miR-98, miR-125b-1*, and let-7e* as new potential diagnostic biomarkers in ulcerative colitis, World J. Gastroenterol. 19 (2013) 4289–4299.
[26] M. Iborra, F. Bernuzzi, C. Correale, S. Vetrano, G. Fiorino, B. Beltran, F. Marabita, M. Locati, A. Spinelli, P. Nos, P. Invernizzi, S. Danese, Identification of serum and tissue micro-RNA expression profiles in different stages of inflammatory bowel disease, Clin. Exp. Immunol. 173 (2013) 250–258.
[27] L. Zou, X. Xiong, K. Wang, Y. Yin, MicroRNAs in the intestine: role in renewal, homeostasis, and inflammation, Curr. Mol. Med. 18 (2018) 190–198.
[28] L.C. Yu, A.N. Flynn, J.R. Turner, A.G. Buret, SGLT-1-mediated glucose uptake protects intestinal epithelial cells against LPS-induced apoptosis and barrier defects: a novel cellular rescue mechanism? FASEB J. 19 (2005) 1822–1835.
[29] P. Akbari, S. Braber, A. Alizadeh, K.A. Verheijden, M.H. Schoterman, A.D. Kraneveld, J. Garssen, J. Fink-Gremmels, Galacto-oligosaccharides protect the intestinal barrier by maintaining the tight junction network and modulating the inflammatory responses after a challenge with the mycotoxin deoxynivalenol in human Caco-2 cell monolayers and B6C3F1 mice, J. Nutr. 145 (2015) 1604–1613.
[30] K.J. Livak, T.D. Schmittgen, Analysis of relative gene expression data using real-time quantitative PCR and the 2(−Delta Delta C(T)) method, Methods 25 (2001) 402–408.
[31] L.Y. Wu, C.C. Juan, L.T. Ho, Y.P. Hsu, L.S. Hwang, Effect of green tea supplementation on insulin sensitivity in Sprague-Dawley rats, J. Agric. Food Chem. 52 (2004) 643–648.
[32] A. Gopalakrishnan, J.F. Clinthorne, E.A. Rondini, S.J. McCaskey, E.A. Gurzell, I.M. Langohr, E.M. Gardner, J.I. Fenton, Supplementation with galacto-oligosaccharides increases the percentage of NK cells and reduces colitis severity in Smad3-deficient mice, J. Nutr. 142 (2012) 1336–1342.
[33] M.D.M. Rigo-Adrover, K. Knipping, Prevention of rotavirus diarrhea in suckling rats by a specific fermented Milk concentrate with prebiotic mixture, 11 (2019).
[34] F. Wu, N.J. Guo, H. Tian, M. Marohn, S. Gearhart, T.M. Bayless, S.R. Brant, J.H. Kwon, Peripheral blood microRNAs distinguish active ulcerative colitis and Crohn's disease, Inflamm. Bowel Dis. 17 (2011) 241–250.
[35] A. Paraskevi, G. Theodoropoulos, I. Papaconstantinou, G. Mantzaris, N. Nikiteas, M. Gazouli, Circulating MicroRNA in inflammatory bowel disease, J Crohns Colitis 6 (2012) 900–904.
[36] L.B. McKenna, J. Schug, A. Vourekas, J.B. McKenna, N.C. Bramswig, J.R. Friedman and K.H. Kaestner, MicroRNAs control intestinal epithelial differentiation, architecture, and barrier function, Gastroenterology 139 (2010), 1654-1664, 1664.e1.
[37] D. Ye, S. Guo, R. Al-Sadi, T.Y. Ma, MicroRNA regulation of intestinal epithelial tight junction permeability, Gastroenterology 141 (2011) 1323–1333.
[38] F.J. Moreno, A. Montilla, M. Villamiel, N. Corzo, A. Olano, Analysis, structural characterization, and bioactivity of oligosaccharides derived from lactose, Electrophoresis 35 (2014) 1519–1534.
[39] G. Leforestier, A. Blais, F. Blachier, A. Marsset-Baglieri, A.M. Davila-Gay, E. Perrin, D. Tome, Effects of galacto-oligosaccharide ingestion on the mucosa-associated mucins and sucrase activity in the small intestine of mice, Eur. J. Nutr. 48 (2009) 457–464.
[40] M.A. Bouwhuis, M.J. McDonnell, T. Sweeney, A. Mukhopadhya, C.J. O'Shea, J.V. O'Doherty, Seaweed extracts and galacto-oligosaccharides improve intestinal health in pigs following Salmonella typhimurium challenge, Animal 11 (2017) 1488–1496.
[41] I. Yang, D. Eibach, F. Kops, B. Brenneke, S. Woltemate, J. Schulze, A. Bleich, A.D. Gruber, S. Muthupalani, J.G. Fox, C. Josenhans, S. Suerbaum, Intestinal microbiota composition of interleukin-10 deficient C57BL/6J mice and susceptibility to Helicobacter hepaticus-induced colitis, PLoS One 8 (2013) e70783.
[42] K. Takeda, G. Dennert, The development of autoimmunity in C57BL/6 lpr mice correlates with the disappearance of natural killer type 1-positive cells: evidence for their suppressive action on bone marrow stem cell proliferation, B cell immunoglobulin secretion, and autoimmune symptoms, J. Exp. Med. 177 (1993) 155–164.
[43] M.M. Fort, M.W. Leach, D.M. Rennick, A role for NK cells as regulators of CD4+ T cells in a transfer model of colitis, J. Immunol. 161 (1998) 3256–3261.
[44] D.D. Eichele, K.K. Kharbanda, Dextran sodium sulfate colitis murine model: an indispensable tool for advancing our understanding of inflammatory bowel diseases pathogenesis, World J. Gastroenterol. 23 (2017) 6016–6029.
[45] A. Soroosh, M. Koutsioumpa, C. Pothoulakis, D. Iliopoulos, Functional role and therapeutic targeting of microRNAs in inflammatory bowel disease, Am. J. Physiol. Gastrointest. Liver Physiol. 314 (2018) G256–g262.
[46] B. Chen, S. She, D. Li, Z. Liu, X. Yang, Z. Zeng, F. Liu, Role of miR-19a targeting TNF-alpha in mediating ulcerative colitis, Scand. J. Gastroenterol. 48 (2013) 815–824.
[47] P. Zhou, B. Chen, P. Hu and Y. Sun, [Role of miR-19a in ulcerative colitis in mice], Nan Fang Yi Ke Da Xue Xue Bao 33 (2013), 1325–8.