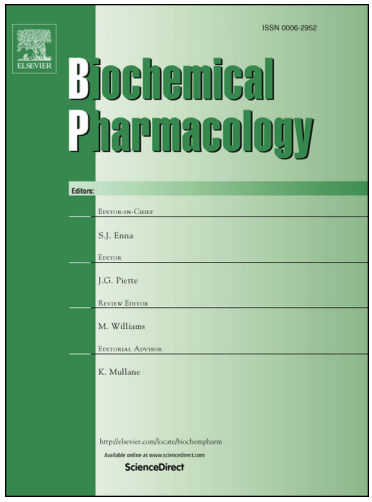
Accepted Manuscript



IL-10/microRNA-155/SHIP-1 signaling pathway is crucial for commensal bacteria induced spontaneous colitis

Yi Li, Yun Tian, Weiming Zhu, Jianfeng Gong, Zhen Guo, Feilong Guo, Lili Gu, Jieshou Li

PII: S0006-2952(16)30167-8
DOI: http://dx.doi.org/10.1016/j.bcp.2016.07.002
Reference: BCP 12582

To appear in: *Biochemical Pharmacology*

Received Date: 2 May 2016
Accepted Date: 5 July 2016

Please cite this article as: Y. Li, Y. Tian, W. Zhu, J. Gong, Z. Guo, F. Guo, L. Gu, J. Li, IL-10/microRNA-155/SHIP-1 signaling pathway is crucial for commensal bacteria induced spontaneous colitis, *Biochemical Pharmacology* (2016), doi: http://dx.doi.org/10.1016/j.bcp.2016.07.002

Title: **IL-10/microRNA-155/SHIP-1 signaling pathway is crucial for commensal bacteria induced spontaneous colitis**

Short title: **IL-10/MiR-155/SHIP-1 signaling in IBD**

Authors: Yi Li,[1,†] M.D., Ph.D., Yun Tian,[2,†] M.D., Ph.D., Weiming Zhu,[1,*] M.D., Ph.D., Jianfeng Gong,[1] M.D., Ph.D., Zhen Guo,[1] M.D., Ph.D., Feilong Guo,[1] M.S., Lili Gu,[1] M.D., Ph.D., Jieshou Li,[1] M.D.

Affiliation: 1 Department of General Surgery, Jinling Hospital, Medical School of Nanjing University, No. 305 East Zhongshan Road, Nanjing, PR China

2 Department of Oncology, the Second Affiliated Hospital of Nanjing Medical University, No. 121 Jiangjiayuan Road, Nanjing, PR China

[†] The first two authors contributed equally to this work.

Address correspondence and reprint requests to:

Weiming Zhu, MD.

Department of General surgery, Jinling Hospital, Medical School of Nanjing University

305 East Zhongshan Road, Nanjing 210002, China.

Tel.: +86 25 84806839; fax: +86 25 84806839. Email: juwiming@nju.edu.cn

Author contributions:

Y.L., Y.T. and W-M.Z. conceived and designed the study, acquisition and interpretation of data and were involved in drafting of the manuscript; Y.L., Y.T., J.G., and F.G. were involved analysis, acquisition and interpretation of data; Z.G. and L.G. were involved in acquisition of data; W-M.Z. and J.L. were involved critical revision of the manuscript for important intellectual content and were involved in study concept and design.

ABSTRACT

Interleukin 10 (IL-10) microRNA-155 (miR-155)/ Src homology 2 domain–containing inositol 5-phosphatase 1 (SHIP-1) signaling pathway plays an important role in maintaining immune homeostasis. We aimed to determine characterize the changes induced by commensal bacteria on the IL-10/miR-155/SHIP-1 signaling pathway, as well as the potential therapeutic effects of anti-miR-155 on colitis in IL-10 deficient (IL-10$^{-/-}$) mice. Age- and sex-matched C57BL/6 IL-10$^{-/-}$ and wild type mice were transferred from a germ-free environment to a specific pathogen free condition. Part of IL-10$^{-/-}$ mice were then treated with anti-miR-155. IL-10/miR-155/SHIP-1 signaling pathway was evaluated and the therapeutic effects of anti-miR-155 treatment on colitis in IL-10$^{-/-}$ mice was assessed. The expression and the relationship of IL-10, miR-155, and SHIP-1 were also measured in patients with active Crohn's colitis. IL-10/miR-155/SHIP-1 signaling pathway was activated in IL-10$^{-/-}$ mice transferring from a germ-free environment to a specific pathogen free condition. Anti-miR-155 treatment significantly ameliorated the severity of colitis in IL-10$^{-/-}$ mice. Additionally, administration of anti-miR-155 was associated with a restoration of SHIP-1 signaling pathway. The relationship of IL-10, miR-155, and SHIP-1 was confirmed in human study using samples from patients with active Crohn's colitis. IL-10/miR-155/SHIP-1 pathways plays a critical role in commensal bacteria induced colitis and miR-155 may be a potential therapeutic target for human inflammatory bowel disease.

KEYWORDS

Crohn's disease; IL-10; MicroRNA-155; SHIP-1; Commensal bacteria

ABBREVIATIONS

CD, Crohn's disease; GF, germ-free; IBD, inflammatory bowel disease; IL-10, interleukin-10; IL-10$^{-/-}$, IL-10 deficient; miR-155, microRNA-155; SD, standard deviation; SPF, specific pathogen-free; SHIP-1, Src homology 2 domain–containing inositol 5-phosphatase 1; Tregs, regulatory T cells; UC, ulcerative colitis.

1. INTRODUCTION

Inflammatory bowel disease (IBD), encompassing the chronic relapsing inflammatory disorders such as Crohn's disease (CD) and ulcerative colitis (UC), is thought to result from an aberrant immune response to intestinal microbiota in genetically susceptible individuals.[1, 2] Recent evidence from bench research including genetics, immunology, microbiology, experimental animal models and clinical studies, demonstrate the abnormal host-microbial interactions in the pathogenesis of IBD.[3]

The microbiota in the gut contribute to host nutrition and interact with the host immune system.[4] In addition to pathogenic microbes, otherwise harmless commensal microbes can also trigger the inflammatory diseases of the intestine such as IBD in some distinct circumstance,[5] which might be explained by the concept that intestinal inflammation of intestine may arise from lack of tolerance to antigens present in autologous microflora in genetically susceptible host.[6] Studies of experimental models have shown that the intestinal microbiota play an important role in the development of chronic intestinal inflammation.[7-9] In humans, diversion of the fecal stream could lead to improvement of downstream inflammation, while postoperative exposure of the intestine to luminal contents results in inflammation, implying the involvement of intestinal microbiota in the development of IBD.[10, 11] In addition, in patients with IBD, although routine use is limited by the development of antibiotic resistance and systemic side effects, antibiotics show therapeutic activity.[12-14]

In blood samples from IBD patients, microRNA-155 (miR-155) is found to be highly expressed.[15] With an emerging role in regulating immune responses, in addition to regulating the development of regulatory T cells (Tregs), miR-155 is associated with inflammation and autoimmunity through controlling the differentiation of $CD4^+$ T cells into the Th1, Th2 and Th17 subsets of helper T cells.[16] MiR-155 has also been shown to directly suppress the expression of Src homology 2 domain–containing inositol 5-phosphatase 1 (SHIP-1),[17, 18] a negative

regulator in a variety of cytokine, immunoreceptor, and growth factor signaling pathways.[19-21] The role of SHIP-deficient mice develop segmental ilietis with classical features of CD.[22]

The anti-inflammatory cytokine interleukin-10 (IL-10) has been identified as being involved in IBD [23] including UC and CD.[24, 25] In vivo, IL-10 deficient (IL-10$^{-/-}$) mice develop transmural inflammation of the intestine under standard housing condition but remain free of intestinal inflammation in sterile germ-free (GF) conditions.[8] IL-10 is thought, at least in part, to act through controlling miR-155 expression.[26] IL-10, which is an anti-inflammatory cytokine secreted by a variety of cell types and is critical for maintaining immune homeostasis in the gastrointestinal tract,[27] controls the level of functional miR-155.[28, 29] The expression of miR-155 is found to be inhibited by IL-10 and this inhibitory effect lead to an increase in the expression of the miR-155 target, SHIP-1.[30] Indeed, the close positive correlation of SHIP-1 expression with IL-10 expression, has also been found in patients with IBD.[31]

The goals of the current study was to characterize the changes induced by commensal bacteria in the IL-10/miR-155/SHIP-1 signaling pathway, as well as the potential therapeutic effects of anti-miR-155 on colitis in IL-10$^{-/-}$ mice shifted from GF to specific pathogen-free (SPF) environment. In this study, we report that IL-10 is essential for the bacterial flora induced colitis and the activation of miR-155 signaling pathway in mice. In addition, anti-miR-155 treatment attenuated the severity of colitis. Our study provided evidence that IL-10/miR-155/SHIP-1 pathways plays a critical role in commensal bacteria induced colitis and miR-155 may be an intriguing therapeutic target for human IBD.

2. MATERIALS AND METHODS

2.1. Ethics Statements

For studies using clinical human materials, informed consent was obtained from all participants and the approval from the Research Ethics Committee of Jinling Hospital (Nanjing,

China) was obtained. All mice received humane care in accordance with the law concerning the protection and control of animals in China.

2.2. Animal and experimental design

Age- and sex-matched C57BL/6 IL-10$^{-/-}$ and wild type mice were obtained from the Jackson Laboratory (Bar Harbor, Maine) and maintained in a GF animal facility at the Model Animal Research Center of Nanjing University (Nanjing, China). At 4 weeks of age, part of IL-10$^{-/-}$ and WT mice were transferred to a SPF condition. Mice were kept at a constant temperature with a 12-h light/dark cycle. Littermates were not used in this study.

IL-10$^{-/-}$ mice under SPF condition were treated with anti-miR-155 or normal saline 4 weeks after transferring. An antisense oligonucleotide modified by locked nucleotide acid (LNA; Exiqon) was synthesized to inhibit miR-155 as described by Murugaiyan et al.[32] For in vivo anti–miR-155 treatment, 30 μl lipofectamine 2000 (Invitrogen) was mixed with anti–miR-155 (20 mg/mouse) dissolved in 170 ml PBS, and the liposome complexes were intraperitoneal injected every other day for 4 weeks. Sacrifice was performed when mice were 12 weeks old. Tissue samples were obtained and fixed or stored at −80◦C until assay. Colon length and colon weight/length ratio was recorded.

2.3. Histopathology

The colons of IL-10$^{-/-}$ mice treated with or without anti- miR-155 were harvested and fixed in Bouin's fixative. As previously described,[33] these tissues were paraffin-embedded, sectioned, and stained with hematoxylin and eosin. Tissues were reviewed in a blinded fashion and assessed according to a previously validated intestinal histological inflammatory score described by Madsen et al,[34] with the histological scores ranged from 0 to 10.

2.4. Patients and Tissue Specimens

Colonic samples were obtained from ascending colon in patients with Crohn's colitis and non-IBD control subjects. The diagnosis CD was based on conventional clinical, endoscopic, and histopathological criteria.[35] Colonic samples were collected from CD patients with active colitis (Crohn's disease activity index > 150) who were not exposure to steroids, immunosuppressive drugs or biological agents during the recent 30 days. The tissue samples were collected and stored at −80 ◦C until assay.

2.5. RT-PCR

Total DNA was extracted from the mucosal tissue from patients with Crohn's colitis and IL-10$^{-/-}$ mice. The first-strand cDNA synthesis were performed as described previously.[33, 36] The gene expressions of miR-155, SHIP-1, and IL-10 were evaluated. Quantitative real-time PCR with SYBR green PCR Master Mix (Applied Biosystems, Foster City, CA, USA) was performed in a MicroAmp Fast Optical 96-Well Reaction Plate (Applied Biosystems) using the 7300 Fast Real-Time PCR System (Applied Biosystems) for thermal cycling and real-time fluorescence measurements. Positive and negative controls were included in all the experiments and each sample was run in triplicate for each PCR. All PCR reactions were done in triplicate and product amplification results were normalized to β-actin expression for each sample. Relative fold changes in RNA levels were calculated by the ΔΔCT method. The range for the target, relative to a calibrator sample was calculated by $2^{-\Delta\Delta CT}$.

2.6. Western blot analysis

As previously described,[36-38] the protein expression of SHIP-1, NF-κB p65, Akt and p-Akt was assessed in colon mucosal tissue homogenizing in 2-3 ml of lysis buffer with a protease inhibitor cocktail (Sigma Chemical, St. Louis, MO, USA). Extracted protein was separated on a 10% denaturing polyacrylamide gel and transferred to a polyvinylidene difluoride membrane by electroblotting at 4 ◦C. Nuclear extracts were prepared using NE-PER nuclear and

cytoplasmic extraction reagents to detect the nuclear level of NF-κB (Pierce, Rockford, IL, USA). Transferred membranes were then blocked for 2 h at RT with 5% non-fat dried milk in TBST before incubating with anti-mouse antibodies (Santa Cruz Biotechnology) at 1:500 dilution in blocking buffer overnight at 4 °C. After washing with TBST, the membranes were incubated with a peroxidase-conjugated secondary antibody, diluted at 1:10000 in blocking buffer, for 1 h at RT. Detection was performed by incubating the membranes with ECL Plus (AMRESCO, USA) and exposed to X-ray films. The expression of Akt and p-Akt was relative to β/Actin, while the level of NF-κB p65 protein and SHIP-1 was statistically analyzed relative to Histone H3.1and α-Tubulin respectively.

2.7. ELISA for the secretion of cytokines and MPO

Levels of IFN-γ, TNF-α, MPO were measured using enzyme-linked immunosorbent assay (ELISA) kits (R& D Systems, Minneapolis, MN, USA) according to the manufacturer's instructions.

2.8. Statistical Analyses

All data are expressed as the mean ±standard deviation (SD). Statistical significance of evaluated data was tested using Mann-Whitney's U-test and Student's t-test. Correlation between groups was assessed using the Pearson's test. P-values less than 0.05 were considered to be significant.

3. RESULTS

3.1. MiR-155 signaling pathway was activated in IL-10$^{-/-}$ mice by commensal bacteria

After transferring to SPF conditions for 4 weeks, the level of miR-155 was greater in IL-10$^{-/-}$ than that in SPF WT mice. In addition, significant difference was observed in terms of miR-155 in WT mice under SPF conditions and IL-10$^{-/-}$ mice in GF circumstance. Interestingly, the expression of miR-155 was significantly increased in IL-10$^{-/-}$ mice under SPF conditions when compared with that in GF condition (Figure 1A). This resulted in a lower expression of SHIP-1 protein in IL-10$^{-/-}$ mice than WT mice, in SPF conditions. As expected, the expression of SHIP-1 was decreased in GF IL-10$^{-/-}$ mice when compared to SPF WT mice. Additionally, significant difference in the SHIP-1 level was found between IL-10$^{-/-}$ mice in SPF conditions and those in GF environments (Figure 1B). These results confirmed the theory that IL-10 was required for miR-155 inhibition. Furthermore, even absence the IL-10, the miR-155 would not activated in germ free condition, suggesting commensal bacterial was necessary for this signaling activation.

3.2. Anti-miR-155 attenuated the colitis in IL-10$^{-/-}$ mice

We then investigated whether systemic administration of anti-miR-155 in vivo attenuates the intestinal inflammation in SPF IL-10$^{-/-}$ mice. The colon was significantly longer in IL-10$^{-/-}$ mouse receiving anti-miR-155 treatment (Figure 2A). In addition, another intestinal inflammatory parameter, colon weight, was significantly reduced in mice with the administration of anti-miR-155 (Figure 2B). Administration of LNA-modified anti-miR-155 was also associated with dramatically decreased histological score in SPF IL-10$^{-/-}$ mice (Figure 2C and 2D). Additionally, we found that administration of anti-miR-155 reduced the secretion of cytokines including MPO (Figure 3A), TNF-α and IFN-γ (Figure 3B).

3.3. Therapeutic effects of anti-miR-155 in IL-10$^{-/-}$ mice was associated with increased SHIP-1

SHIP, which plays an important role in immune modulation, limits the activation of key downstream pathways such as Akt and NF-kB. Our preliminary data indicated that, in SPF WT mice, silence of miR-155 significantly increased the expression of SHIP-1 (data are not shown).

To investigate whether anti-miR-155 treatment affected SHIP-1 and its target signaling pathway, we evaluated the expression of ship signaling in mice with colitis treated with or without anti-miR-155. We found that colon mucosa from anti-miR-155-treated SPF IL-10$^{-/-}$ mice had an increased SHIP-1 expression (Figure 4A and 4B), inhibited NF-κB activation (Figure 4C and 4D). In addition, we found that anti-miR-155 treatment prevented upregulation of p-Akt (Figure 4E and 4F). Thus, the therapeutic effects of anti-miR-155 in intestinal inflammation in SPF IL-10$^{-/-}$ mice was association with an increased expression of SHIP-1 and a modulation of its target signaling pathway.

3.4. The relationship of IL-10, miR-155 and SHIP-1 in patients with Crohn's colitis

The role of IL-10/miR-155/SHIP-1 signaling played in clinical CD is still uncovered. Therefore, it is important to investigate the expression and the relationship of IL-10, miR-155, and SHIP-1. By using quantitative reverse transcription PCR, we investigated the gene expression of microRNA-155, SHIP-1 and IL-10 in mucosal biopsies from 20 patients with active Crohn's colitis and 18 non-IBD control subjects (Figure 5A). Interestingly, the expression of IL-10 messenger RNA was inversely correlated with miR-155 level (Figure 5B), suggesting that IL-10 may be involved in the regulation of miR-155. Similarly, a significant correlation was confirmed between miR-155 RNA levels and SHIP-1expression (Figure 5C), implying SHIP-1 is the target of miR-155. In addition, a strong positive correlation was found between IL-10 and SHIP-1 mRNA levels (Figure 5D), which suggested SHIP-1 could be regulated by IL-10. However, no correlation was found in non-IBD control subjects in respect to the relationship of IL-10, miR-155 and SHIP-1 (Figure 5B, C, and D). We also evaluated the relationship between IL-10, miR-155, SHIP-1 and disease activity which was indicated as CDAI. However, no correlation was found between the expression of miR-155 and CDAI ($r=0.187$, $p=0.429$). In addition, there was no significant correlation between IL-10 ($r=0.202$, $p=0.395$), SHIP-1 ($r=0.071$, $p=0.760$) and CDAI.

4. DISCUSSION

IL-10/miR-155/SHIP-1 has been demonstrated to be an important regulator in the response to microorganism products.[26, 28, 30] The present study showed a development of colitis and activated miR-155 signaling pathway in animals shifted from GF to a SPF environment. A major objective of the present study was to explore the role played by IL-10/miR-155/SHIP-1 in the commensal bacterial induced animal colitis. Indeed, SPF IL-10$^{-/-}$ mice developed spontaneous colitis while those in GF did not.[8] In addition, the altered expression of miR-155 and SHIP-1 in IL-10$^{-/-}$ mice suggests that miR-155/SHIP-1 signaling pathway plays an important role in commensal bacterial induced colitis. Furthermore, the relationship of IL-10, miR-155, and SHIP-1 was demonstrated in patients with active Crohn's colitis.

The currently accepted hypothesis is that commensal bacteria resident in gut are essential for the development of chronic intestinal inflammation in genetically susceptible hosts. This notion is primarily supported by the fact that commensal enteric bacterial antigens continuously induce perpetual colitis.[8, 39-42] The ability of antibiotics to decrease postoperative recurrence of CD suggests that intestinal microbial flora is important for the initiation and perpetuation of human IBD.[43, 44] This is confirmed by a study indicating that recurrent disease is found after reinfusion of luminal contents in patients with fecal diversion.[10] Indeed, the present study demonstrates that chronic colitis cannot develop in IL-10$^{-/-}$ mice under GF conditions but in SPF environment. Therefore, our study confirm the important role played by the commensal flora in triggering and exacerbating chronic intestinal inflammation in this IBD animal model. However, it is still unclear how the microbiota initiates the inflammatory response and, thereby, contributes to the chronic colitis. In this study, we found a dysregulated miR-155/SHIP-1 pathway in SPF IL-10$^{-/-}$ mice compared with those housed in GF conditions.

To further explore the role of miR-155/SHIP pathway in IL-10$^{-/-}$ mice with commensal bacteria induced colitis, we observed the effects of miR-155 inhibition on this animal model. In our study, IL-10$^{-/-}$ mice developed colitis and had an increased secretion of cytokines, after shifting from GF to SPF conditions. In these mice, the length of colon was decreased. Moreover, another parameter of colitis, the weight of colon, was significantly increased. Interestingly, anti-miR-155 treatment attenuated the colitis, which was confirmed by the reduced colon length and decreased colon weight. Both histological score and the cytokines secretion were decreased in mice receiving anti-miR-155 treatment, suggestingthe acceptable therapeutic effects of anti-miR-155 in this IBD model.

Microbiota is not only important in controlling host intestinal immune homeostasis,[45] in addition, it also regulates the process of intestinal inflammation although the mechanisms involving regulation of mucosal immunity are only incompletely understood.[46, 47] In the current study, we found that microbiota upregulated mucosal miR-155 expression in mice with IL-10$^{-/-}$ mice. Anti-miR-155 treatment inhibited the mucosal miR-155 and, thus, could increase the expression of SHIP-1 and regulation its target signaling. Our study has its limitations such as the littermates were not used and the enteric microbiota were not evaluated, given that altered enteric microbiota was found in IL-10-/- mice during development of colitis[48] and miR-155 expression are modulated by microbial ligands.[49] However, since all IL-10-/- mice were housed in the same SPF condition, the impact of luminal bacterial load and the specific bacteria strains on the intestinal inflammation as well as miR-155 signaling is limited. Importantly, the aberrant IL-10/miR-155/SHIP-1 signaling pathway was also demonstrated in patients with Crohn's colitis. By using samples from patients, our study demonstrated a positive correlation between IL-10 and SHIP-1. In contrast, a reverse correlation between IL-10 and miR-155 was found in patients with Crohn's colitis, suggesting a pathogenesis role of IL-10/miR-155/SHIP-1 in the development of IBD.

In summary, our data demonstrated that microbiota regulates miR-155/SHIP-1 pathway in IL-10$^{-/-}$ mice. Through regulation of mucosal expression SHIP-1 and its target in response to microbiota stimulation, anti-miR-155 shows a therapeutic effect on colitis in IL-10$^{-/-}$ mice. Given the ameliorating effect of anti-miR-155 in IL-10$^{-/-}$ mice with colitis and the relationship of IL-10-miR-155/SHIP-1in Crohn's patients, silencing miR-155 may be an effective therapeutic approach in the treatment of IBD.

Acknowledgements: This work was partly supported by National Natural Science Foundation of China (Grant 81200263, 81302110, and 81570500).

Disclosure: The authors declare no conflict of interest.

FIGURE LEGEND

Figure 1 MiR-155/SHIP-1 signaling was activated in IL-10$^{-/-}$ mice by commensal bacteria. The relative expression of miR-155 (A) and SHIP-1 (B and C) in mucosal tissue. Data expressed as means ± SD (n=10 per group). $^{*}P < 0.05$.

Figure 2 Anti-miR-155 ameliorated the colitis in IL-10$^{-/-}$ mice. Anti-miR-155 treatment significantly increased the colon length (A) and decreased colon weights of IL-10-/- mice (B). The inflammation in the colon was restored (C) . Marked infiltrations of mononuclear cells and polymorphonuclear cells, focal mucosal ulcerations, crypt abscess and epithelial cell hypertrophy were observed in colonic mucosa of IL-10-/- mice (Figure 2C-a). In contrast, anti-miR-155-treated mice showed significantly less infiltration of these cells and attenuated histological appearance of the mucosa and submucosa (Figure 2C-b). The colonic inflammation score was decreased in the IL-10-/- mice treated with anti-miR-155 (D). Data expressed as means ± SD (n=10 per group). $^{*}P < 0.05$.

Figure 3 Anti-miR-155 reduces chronic colonic inflammation in IL-10$^{-/-}$ mice. The concentration of MPO (A), IFN-γ and TNF-α (B) in the colonic mucosal tissue were significantly decreased in Anti-miR-155-treated IL-10$^{-/-}$ mice. Data expressed as means ± SD (n=10 per group). $^{*}P < 0.05$.

Figure 4 Therapeutic effects of anti-miR-155 in IL-10$^{-/-}$ mice was associated modulation of SHIP-1 pathway. An increased expression of SHIP-1 (A and B), a reduced activity of NF-κB (C and D) , and an reduced expression of P-Akt (E and F) was found IL-10$^{-/-}$ mice receiving anti-miR-155. Data expressed as means ± SD (n=10 per group). $^{*}P < 0.05$.

Figure 5 The relationship of IL-10, miR-155 and SHIP-1 in patients with active Crohn's colitis. Relative mRNA expression of IL-10, miR-155 and SHIP-1 (A). Correlation between IL-10 and miRNA-155 mRNA (B), miRNA-155and SHIP-1 mRNA (C), and IL-10 and SHIP-1 mRNA (D) in patients with CD. Data expressed as means ± SD (n=19).

REFERENCES

[1] Kaser A, Zeissig S, Blumberg RS. Inflammatory bowel disease. Annu Rev Immunol 2010;28:573-621.

[2] Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature 2007;448:427-34.

[3] Sartor RB, Blumberg RS, Braun J, Elson CO, Mayer LF. CCFA microbial-host interactions workshop: highlights and key observations. Inflamm Bowel Dis 2007;13:600-19.

[4] Littman DR, Pamer EG. Role of the commensal microbiota in normal and pathogenic host immune responses. Cell Host Microbe 2011;10:311-23.

[5] Arumugam M, Raes J, Pelletier E, Le Paslier D, Yamada T, Mende DR, et al. Enterotypes of the human gut microbiome. Nature 2011;473:174-80.

[6] Duchmann R, Neurath MF, Meyer zum Buschenfelde KH. Responses to self and non-self intestinal microflora in health and inflammatory bowel disease. Res Immunol 1997;148:589-94.

[7] Garrett WS, Lord GM, Punit S, Lugo-Villarino G, Mazmanian SK, Ito S, et al. Communicable ulcerative colitis induced by T-bet deficiency in the innate immune system. Cell 2007;131:33-45.

[8] Kuhn R, Lohler J, Rennick D, Rajewsky K, Muller W. Interleukin-10-deficient mice develop chronic enterocolitis. Cell 1993;75:263-74.

[9] Schultz M, Tonkonogy SL, Sellon RK, Veltkamp C, Godfrey VL, Kwon J, et al. IL-2-deficient mice raised under germfree conditions develop delayed mild focal intestinal inflammation. Am J Physiol 1999;276:G1461-72.

[10] D'Haens GR, Geboes K, Peeters M, Baert F, Penninckx F, Rutgeerts P. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. Gastroenterology 1998;114:262-7.

[11] Rutgeerts P, Goboes K, Peeters M, Hiele M, Penninckx F, Aerts R, et al. Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. Lancet 1991;338:771-4.

[12] Khan KJ, Ullman TA, Ford AC, Abreu MT, Abadir A, Marshall JK, et al. Antibiotic therapy in inflammatory bowel disease: a systematic review and meta-analysis. Am J Gastroenterol 2011;106:661-73.

[13] Ohkusa T, Kato K, Terao S, Chiba T, Mabe K, Murakami K, et al. Newly developed antibiotic combination therapy for ulcerative colitis: a double-blind placebo-controlled multicenter trial. Am J Gastroenterol 2010;105:1820-9.

[14] Sartor RB. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. Gastroenterology 2004;126:1620-33.

[15] Paraskevi A, Theodoropoulos G, Papaconstantinou I, Mantzaris G, Nikiteas N, Gazouli M. Circulating MicroRNA in inflammatory bowel disease. J Crohns Colitis 2012;6:900-4.

[16] Seddiki N, Brezar V, Ruffin N, Levy Y, Swaminathan S. Role of miR-155 in the regulation of lymphocyte immune function and disease. Immunology 2013.

[17] Costinean S, Sandhu SK, Pedersen IM, Tili E, Trotta R, Perrotti D, et al. Src homology 2 domain-containing inositol-5-phosphatase and CCAAT enhancer-

binding protein beta are targeted by miR-155 in B cells of Emicro-MiR-155 transgenic mice. Blood 2009;114:1374-82.

[18] O'Connell RM, Chaudhuri AA, Rao DS, Baltimore D. Inositol phosphatase SHIP1 is a primary target of miR-155. Proc Natl Acad Sci U S A 2009;106:7113-8.

[19] Daigle I, Yousefi S, Colonna M, Green DR, Simon HU. Death receptors bind SHP-1 and block cytokine-induced anti-apoptotic signaling in neutrophils. Nat Med 2002;8:61-7.

[20] Freeburn RW, Wright KL, Burgess SJ, Astoul E, Cantrell DA, Ward SG. Evidence that SHIP-1 contributes to phosphatidylinositol 3,4,5-trisphosphate metabolism in T lymphocytes and can regulate novel phosphoinositide 3-kinase effectors. J Immunol 2002;169:5441-50.

[21] Ono M, Bolland S, Tempst P, Ravetch JV. Role of the inositol phosphatase SHIP in negative regulation of the immune system by the receptor Fc(gamma)RIIB. Nature 1996;383:263-6.

[22] Kerr WG, Park MY, Maubert M, Engelman RW. SHIP deficiency causes Crohn's disease-like ileitis. Gut 2011;60:177-88.

[23] Franke A, Balschun T, Karlsen TH, Sventoraityte J, Nikolaus S, Mayr G, et al. Sequence variants in IL10, ARPC2 and multiple other loci contribute to ulcerative colitis susceptibility. Nat Genet 2008;40:1319-23.

[24] Correa I, Veny M, Esteller M, Pique JM, Yague J, Panes J, et al. Defective IL-10 production in severe phenotypes of Crohn's disease. J Leukoc Biol 2009;85:896-903.

[25] Schreiber S, Heinig T, Thiele HG, Raedler A. Immunoregulatory role of interleukin 10 in patients with inflammatory bowel disease. Gastroenterology 1995;108:1434-44.

[26] Quinn SR, Mangan NE, Caffrey BE, Gantier MP, Williams BR, Hertzog PJ, et al. The role of Ets2 transcription factor in the induction of microRNA-155 (miR-155) by lipopolysaccharide and its targeting by interleukin-10. J Biol Chem 2014;289:4316-25.

[27] Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. Annu Rev Immunol 2001;19:683-765.

[28] Chan CS, Ming-Lum A, Golds GB, Lee SJ, Anderson RJ, Mui AL. Interleukin-10 inhibits lipopolysaccharide-induced tumor necrosis factor-alpha translation through a SHIP1-dependent pathway. J Biol Chem 2012;287:38020-7.

[29] Cheung ST, So EY, Chang D, Ming-Lum A, Mui AL. Interleukin-10 inhibits lipopolysaccharide induced miR-155 precursor stability and maturation. PLoS One 2013;8:e71336.

[30] McCoy CE, Sheedy FJ, Qualls JE, Doyle SL, Quinn SR, Murray PJ, et al. IL-10 inhibits miR-155 induction by toll-like receptors. J Biol Chem 2010;285:20492-8.

[31] Arijs I, De Hertogh G, Lemmens B, Van der Goten J, Vermeire S, Schuit F, et al. Intestinal expression of SHIP in inflammatory bowel diseases. Gut 2012;61:956-7.

[32] Murugaiyan G, Beynon V, Mittal A, Joller N, Weiner HL. Silencing microRNA-155 ameliorates experimental autoimmune encephalomyelitis. J Immunol 187:2213-21.

[33] Li Y, Zuo L, Zhu W, Gong J, Zhang W, Guo Z, et al. Telmisartan attenuates the inflamed mesenteric adipose tissue in spontaneous colitis by mechanisms involving regulation of neurotensin/microRNA-155 pathway. Biochem Pharmacol 93:461-9.

[34] Madsen KL, Doyle JS, Jewell LD, Tavernini MM, Fedorak RN. Lactobacillus species prevents colitis in interleukin 10 gene-deficient mice. Gastroenterology 1999;116:1107-14.

[35] Van Assche G, Dignass A, Panes J, Beaugerie L, Karagiannis J, Allez M, et al. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis. J Crohns Colitis 4:7-27.

[36] Li Y, Yu C, Zhu WM, Xie Y, Qi X, Li N, et al. Triptolide ameliorates IL-10-deficient mice colitis by mechanisms involving suppression of IL-6/STAT3 signaling pathway and down-regulation of IL-17. Mol Immunol 47:2467-74.

[37] Li Y, Tian Y, Zhu W, Gong J, Zhang W, Yu C, et al. Triptolide induces suppressor of cytokine signaling-3 expression and promotes lamina propria mononuclear cells apoptosis in Crohn's colitis. Int Immunopharmacol 16:268-74.

[38] Zuo L, Li Y, Wang H, Wu R, Zhu W, Zhang W, et al. Cigarette smoking is associated with intestinal barrier dysfunction in the small intestine but not in the large intestine of mice. J Crohns Colitis 8:1710-22.

[39] Bouma G, Strober W. The immunological and genetic basis of inflammatory bowel disease. Nat Rev Immunol 2003;3:521-33.

[40] Strober W, Fuss IJ, Blumberg RS. The immunology of mucosal models of inflammation. Annu Rev Immunol 2002;20:495-549.

[41] Sartor RB. Mechanisms of disease: pathogenesis of Crohn's disease and ulcerative colitis. Nat Clin Pract Gastroenterol Hepatol 2006;3:390-407.

[42] Strober W, Fuss I, Mannon P. The fundamental basis of inflammatory bowel disease. J Clin Invest 2007;117:514-21.

[43] Rutgeerts P, Hiele M, Geboes K, Peeters M, Penninckx F, Aerts R, et al. Controlled trial of metronidazole treatment for prevention of Crohn's recurrence after ileal resection. Gastroenterology 1995;108:1617-21.

[44] Rutgeerts P, Van Assche G, Vermeire S, D'Haens G, Baert F, Noman M, et al. Ornidazole for prophylaxis of postoperative Crohn's disease recurrence: a randomized, double-blind, placebo-controlled trial. Gastroenterology 2005;128:856-61.

[45] Lee YK, Mazmanian SK. Has the microbiota played a critical role in the evolution of the adaptive immune system? Science 2010;330:1768-73.

[46] Lee YK, Menezes JS, Umesaki Y, Mazmanian SK. Proinflammatory T-cell responses to gut microbiota promote experimental autoimmune encephalomyelitis. Proc Natl Acad Sci U S A 2011;108 Suppl 1:4615-22.

[47] Wu S, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, Yen HR, et al. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. Nat Med 2009;15:1016-22.

[48] Maharshak N, Packey CD, Ellermann M, Manick S, Siddle JP, Huh EY, et al. Altered enteric microbiota ecology in interleukin 10-deficient mice during development and progression of intestinal inflammation. Gut Microbes 4:316-24.

[49] Round JL, O'Connell RM, Mazmanian SK. Coordination of tolerogenic immune responses by the commensal microbiota. J Autoimmun 34:J220-5.