P38 α Deficiency in Macrophages Ameliorates Murine Experimental Colitis by Regulating Inflammation and Immune Process

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Abstract

Introduction: P38 α is a mitogen-activated protein kinase (MAPK) that mediates inflammatory responses. P38 α alterations have been associated with the inflammation-related diseases. However, the role of macrophages-derived p38 α in dextran sulfate sodium (DSS)-induced murine experimental colitis remains unclear.

Objectives: We characterized the role of macrophages-derived p38 α in DSS-induced colitis.

Methods: The expression of macrophage-derived p38 α in human colitis and normal tissues was measured by immunohistochemistry (IHC) and fluorescence in situ hybridization (FISH) analysis. Macrophage-specific p38 α knockout (p38 $\alpha^{\Delta M\phi}$) and wild type (WT) mice administrated by 3% DSS were used to establish experimental colitis. The alterations in inflammatory cytokines, intestinal epithelial barrier, cell proliferation and cell apoptosis between p38 $\alpha^{\Delta M\phi}$ and WT groups were determined by IHC, immunofluorescence (IF), TdT-mediated dUTP Nick-End Labeling (TUNEL) and Western blot analyses. The enriched pathways between p38 $\alpha^{\Delta M\phi}$ and WT groups were identified by RNA-seq and KEGG analysis. SB203580 and BIRB796 as the p38 MAPK inhibitors were used to treat DSS-induced colitis.

Results: p38 α was co-localized with CD68 in the cytoplasm and their co-expression indicated an increased level in colitis tissues as compared with the normal tissues. P38 α deficiency in macrophages was sufficient to suppress the exacerbated clinical symptoms and inflammation responses in experimental colitis, followed by reducing cytokine release, increasing MUC-2 and Claudin-2 secretion and promoting colonic mucosa repair. Further investigations validated that the immune process-related factors such as Lgals9, Rtp4, Ddx60, Nlrp1b, Hsh2d, Oas2 and Oas3 were upregulated in colon tissues from p38 $\alpha^{\Delta M\phi}$ group as compared with the WT group. Inhibition of p38 MAPK attenuated DSS-induced colitis.

Conclusion: Our findings demonstrated that p38 α deficiency in macrophages ameliorated murine experimental colitis by regulating inflammation and immune process.

Introduction

Inflammatory bowel disease (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic and recurrent inflammatory and immune infiltration in gastrointestinal tract (Xavier and Podolsky 2007). Increasing evidence unveils that the pathogenesis of IBD is associated with multiple factors such as genetic polymorphisms, environmental factors, and immunological disorders (Cha, et al. 2017; Shi, et al. 2018). A better understanding of the molecular pathogenesis of IBD is critical to develop promising therapeutic strategies for this disease.

The core of the inflammatory signaling is mediated by reversible phosphorylation by protein kinases, of which mitogen-activated protein kinase (MAPK) signaling acts in linking inflammatory stimuli to cellular responses (Kim, et al. 2008). The p38 MAPKs are a family of serine/threonine protein kinases that consist

of four isoforms p38 α , p38 β , p38 γ and p38 δ (Kumar, et al. 2003a), of which p38 α is the most commonly expressed in human and mouse tissues (Jiang, et al. 1997) and controls inflammatory response and cancer cell proliferation (Hui, et al. 2007; Kumar, et al. 2003a). Hepatocyte-derived p38 α is a pivotal negative regulator in maintaining hepatic gluconeogenesis (Jing, et al. 2015). P38 α appears to have dual roles in certain cancers. It suppresses colitis-associated tumor initiation, but contributes to the proliferation and survival of colon cancer (Gupta, et al. 2014). P38 α deletion in myeloid cells improves experimental colitis, but its deficiency in intestinal epithelial cells (IECs) increases the susceptibility to colitis (Otsuka, et al. 2010).

Emerging evidence indicates that macrophage-specific p38 α accelerates acute liver injury (Liu, et al. 2017) and steatohepatitis progression through inducing pro-inflammatory cytokine secretion and M1 polarization (Zhang, et al. 2019). However, the effects of macrophage-derived p38 α on IBD pathogenesis remain undocumented. In the present study, p38 α was co-localized with CD68 in the cytoplasm and their co-expression displayed an increased level in colitis tissues. We then used macrophage-specific p38 α knockout mice (p38 $\alpha^{\Delta M\phi}$) and WT mice to construct DSS-induced experimental colitis and found that p38 α deficiency in macrophages ameliorated murine experimental colitis by inhibiting cytokine release, promoting colonic mucosa repair and regulating immune process.

Materials And Methods

Tissue sample

10 cases of colitis and 5 normal colon tissues were purchased from Xi'an Best Biotechnology Co., Ltd. (Xi'an, China).

Animals and treatments

C57BL/6 male p38 $\alpha^{\Delta M\phi}$ mice and wild type (WT) littermates (6-8 weeks of age) were provided by Medical College of Xiamen University and housed in the SPF standard laboratory conditions.

As previously described (Weigmann, et al. 2017), p38 $\alpha^{\Delta M\phi}$ mice (n = 5) and WT mice (n = 6) were administrated by the addition of 3% DSS (36–50 kDa, MP Biomedicals, USA) in the drinking water for 7 consecutive days. In another cohort, male C57BL/6 mice (6-8 weeks old) were intraperitoneally injected with p38 MAPK inhibitors or vehicle (DMSO) for 7 consecutive days. According to previous literature (Zhang, et al. 2019), the injection doses of p38 MAPK inhibitors SB203580 and BIRB796 were 50 mg/kg/day and 15 mg/kg/day, respectively. Mouse body weight, colon length, clinical phenotype and pathological scoring were assessed.

Evaluation of DSS-induced colitis

The mice were observed every morning. Weight loss, fecal consistency and intestinal bleeding were recorded in accordance with the previous report (Weigmann, et al. 2017). The DAI value was evaluated

based on the above records.

Histopathological assessment

The colon from the anus to the cecum was obtained and measured. The lower colon tissues in each group were fixed in paraformaldehyde solution (4%) for 48 hours, and then embedded in paraffin. Histopathological assessment of the inflammatory responses in colon tissues in each group was determined by hematoxylin and eosin (H&E) staining in accordance with the previous report (Chen, et al. 2011).

Immunohistochemistry and immunofluorescence

Immunohistochemistry (IHC) and immunofluorescence (IF) analyses were performed to evaluate the protein levels of claudin-2 (AF0128, Affinity Biosciences, USA), MUC-2 (DF8390, Affinity Biosciences, USA), OAS2 ([DF12680](#), Affinity Biosciences, USA), OAS3 (ER1914-27, HuaBio, China), P53 (AF0879, Affinity Biosciences, USA), ki-67 (ab16667, Abcam, United Kingdom), c-myc (AF0358, Affinity Biosciences, USA), TNF- α (17590-1-ap, Proteintech, China), IL-1 β (ab33591, Lianke Biotechnology, China), IL-6 (21865-1-AP, Proteintech, China), and p-p38 (AF4001, Affinity Biosciences, USA), in paraffin-embedded colonic sections in each group. The detailed description of IHC and IF procedure is performed according to the previous reports (Wang, et al. 2017) (Chang, et al. 2019). The sum of the area% was analyzed by ImageJ software.

RNA sequencing and bioinformatic analysis

Briefly, total RNA was extracted from the colon tissues of the WT and p38 $\alpha^{\Delta M\phi}$ groups. The quantitative and quality control of total RNA were detected by NanoDrop ND-1000. 1-2 μ g RNA was extracted from each sample to generate the RNA library, which were sequenced on an Illumina Hiseq 4000 platform after cluster generation. Data were analyzed as follows: sequencing quality control, sequencing data comparison, quantitative analysis of differentially-expressed genes, and GO and KEGG enrichment analysis.

Quantitative Real-time PCR (qRT-PCR)

Trizol reagent (Invitrogen, USA) was used to isolate total RNA from colon tissues. Complementary DNA (cDNA) is obtained by RNA reverse transcription. The reaction procedure is based on our previous reports (Chen, et al. 2019). The $2^{-\Delta\Delta C_t}$ method was used to calculate the gene expression level. GAPDH was used as the internal control. All reactions were performed more than twice. All primers were listed in Table S1.

Western blot analysis

The protein levels of Claudin-2, OAS2 and OAS3 in colon tissues in each group were detected by Western blotting. In briefly, total proteins from colon tissues were obtained with RIPA lysis buffer (Beyotime, Shanghai, China) and protease inhibitor (Servicebio, China). After the determination of protein

concentration, the protein was denatured by boiling water for 15 minutes. The primary antibodies claudin-2 (AF0128, Affinity Biosciences, USA), OAS2 (DF12680, Affinity Biosciences, USA), OAS3 (ER1914-27, HuaBio, China), and anti- β -actin (AF7018, Affinity Biosciences, USA) were thinned and incubated overnight at 4 °C. We added HRP-labeled goat anti-rabbit secondary antibodies in a 1:3000 dilution, and incubated them at room temperature for 30 minutes.

Wash the membranes three times with PBST. The immunoreactive bands were detected with ECL Luminescent Solution (Servicebio, China).

TdT-mediated dUTP Nick-End Labeling (TUNEL) Assay

Apoptosis of IECs was detected by TUNEL kit (Servicebio, China). In short, colon tissue sections were cultured in TUNEL reaction mixture. Then, it was stained with 4', 6-diamino-2-phenylindole (DAPI). After that, all samples were observed under the fluorescence inverted microscope. Each sample was observed in three random fields.

Fluorescence in situ hybridization (FISH) analysis

Oligonucleotide modified probe sequences for P38 α (probe1: 5'-Dig-CGGGCACCTCCCAGATTGTCTTGTT 3', probe2: 5'-Dig-GCCGAGCCAGTCCAAAATCCAGAAT 3', probe3: 5'-Dig-TCCAATACAAGCATCTTCTCCAGC 3') and CD68 (probe1: 5'-Bio-GGGGAATGAGAGAAGCAGGTGGGG 3', probe2: 5'-Bio-GCAATAAGCACCCAGGGCGAGGAGGC 3', Probe3: 5'-Bio-GCCGAGAATGTCCACTGTGCTGCGT 3') were applied for FISH. The detailed operation steps were performed as described above (Wang, et al. 2018). The images were analyzed by pathological image quantitative analysis–HALO software (Indica Labs, USA).

Statistical analysis

Statistical analyses were performed with SPSS 20.0 and GraphPad Prism 8.0.1. All data were displayed as the mean $\pm$ SEM. The differences between groups were analyzed by One-way ANOVA. $P < 0.05$ means statistical difference.

Results

The expression of macrophage-derived p38 α is increased in human colitis tissues

The protein levels of phosphorylated p38 α (p-p38 α) in colitis and normal tissues were detected by IHC analysis, which indicated that, p-p38 α presented no differential expression between colitis and normal tissues ($P=0.1363$, Fig. 1A, B). Further investigations showed that p38 α was co-localized with CD68 in the cytoplasm of colitis tissue cells (Fig. 1C) and they harbored a positive correlation in colitis tissues ($R^2=0.5015$, Fig. 1D). FISH analysis also confirmed that p38 α or CD68 possessed no differential expression between colitis and normal tissues, but the co-expression level of p38 α and CD68 were significantly higher than those in normal tissues ($P=0.0312$ Fig. 1E).

Macrophage-specific knockout of p38 α attenuated murine experimental colitis

To understand how macrophage-derived p38 α leads to the severe colitis, we used p38 $\alpha^{\Delta M\phi}$ and WT mice to establish DSS-induced experimental colitis. The body weight changes, stool consistency, intestinal bleeding and colon length were observed. The results showed that, with or without DSS intervention, the body weight was increased, but DAI was decreased in p38 $\alpha^{\Delta M\phi}$ group as compared with the WT group (Fig. 2A, B). Likewise, after exposure to DSS treatment, the colon length was raised in p38 $\alpha^{\Delta M\phi}$ group as compared with the WT group (Fig. 2C, D). Histological examination of colon sections showed that p38 $\alpha^{\Delta M\phi}$ mice administrated by DSS displayed a lower histopathological scoring in colon tissues accompanied by less neutrophil infiltration, submucosal edema, and loss of crypt and goblet cell as compared with WT mice (Fig. 2E, F).

Macrophage-specific knockout of p38 α inhibits pro-inflammatory cytokine secretion in experimental colitis

To clarify the effects of macrophage-derived p38 α on proinflammatory cytokine production in experimental colitis, we investigated the levels of TNF- α , IL-1 β and IL-6 in p38 $\alpha^{\Delta M\phi}$ and WT groups. Previous studies have indicated that the secretion of cytokines TNF- α , IL-1 β and IL-6 was associated with the pathogenesis of IBD (Hanauer 2006; Souza and Fiocchi 2016). IHC analysis indicated that the expression levels of TNF- α , IL-1 β and IL-6 in experimental colitis in p38 $\alpha^{\Delta M\phi}$ group were significantly lower than those in the WT group (Fig. 3A-C).

Macrophage-specific knockout of p38 α promotes intestinal barrier repair in experimental colitis

Intestinal mucosal integrity can be altered during active colitis, leading to mucosal metabolism disorder and aggravated colon inflammation (Michielan and D'Incà 2015). To understand how p38 α in macrophages affects intestinal barrier function, we tested the tissue levels of mucosal barrier-related markers such as occludin, ZO-1, claudin-1, claudin-2, muc-1 and muc-2 in experimental colitis in p38 $\alpha^{\Delta M\phi}$ and WT groups. qRT-PCR and IHC analysis displayed that occludin, ZO-1, claudin-1 and muc-1 harbored no differential expression (Supplementary Fig. S1), but claudin-2 and muc-2 were upregulated in p38 $\alpha^{\Delta M\phi}$ group as compared with the WT group (Fig. 4A, B). The similar results were further validated by Western blot and IF analyses in p38 $\alpha^{\Delta M\phi}$ and WT groups. (Fig. 4C, D).

Macrophage-specific knockout of p38 α regulates the proliferation and apoptosis of IECs in experimental colitis

Accumulating data show that p38 α is related to cell proliferation in IBD (Otsuka, et al. 2010). To determine the effect of p38 α in macrophages on the proliferation of IECs, ki-67 proliferation index and cell apoptosis in experimental colitis were measured by IHC and TUNEL, which indicated that ki-67 proliferation index of IECs was increased (Fig. 5A), whereas cell apoptosis was decreased in p38 $\alpha^{\Delta M\phi}$ group as compared with the WT group (Fig. 5B).

Macrophage-specific knockout of p38 α enhances immune system process in experimental colitis

To further dissect the underlying mechanisms of macrophage-derived p38 α in experimental colitis, we performed a RNA-seq analysis in experimental colitis between p38 $\alpha^{\Delta M\phi}$ and WT groups. Heatmaps and volcano analysis revealed that 53 differentially-expressed genes were identified between these two groups (Fig. 6A, B). The results of GO analysis revealed that these differential genes were enriched in multiple biological processes, of which immune system process ranked the second place and aroused our attention (Fig. 6C, D). The related genes were shown in Figure 6E. We then validated the expression of these immune system process-related genes between p38 $\alpha^{\Delta M\phi}$ and WT groups by qRT-PCR analysis. The results indicated that the mRNA levels of Lgals9, Rtp4, Ddx60, Nlrp1b, Hsh2d, Oas2 and Oas3 were up-regulated in p38 $\alpha^{\Delta M\phi}$ group compared with the WT group (Fig. 6F). The protein levels of Oas2 and Oas3 were further validated by IHC analysis (Fig. 6G).

The p38 MAPK inhibitor improves DSS-induced experimental colitis

To assess the effects of targeting p38 MAPK on DSS-induced colitis, we used p38 MAPK inhibitors SB203580 and BIRB796 to treat DSS-induced colitis. It was found that the mice in SB203580 group gained an increasement of weight and decline of DAI scoring compared with the DSS group (Fig. 7A, B). Meanwhile, the colon length was increased in SB203580 group as compared with the DSS group (Fig. 7C, D). Histopathological examination demonstrated that SB203580 could inhibit a higher colon pathological scoring in experimental colitis, accompanied by less neutrophil infiltration, loss of crypt and goblet cell, and submucosal edema as compared to with DSS group (Fig. 7E, F).

Discussion

It has been reported that macrophage-derived p38 α promotes liver injury and steatohepatitis progression. Herein, we estimated the role of macrophage-derived p38 α in DSS-induced experimental colitis. We found that the expression of macrophage-derived p38 α was elevated in colitis tissues, and macrophage-specific knockout of p38 α improved the clinical symptoms, DAI and histopathological scoring (Fig. 8). Our findings indicated that macrophage-derived p38 α might be a promising target for colitis.

Accumulating data show that the activation of p38 MAPK signal is involved in intestinal inflammation and responsible for overactive immune system in IBD (Hommes, et al. 2002; ten Hove, et al. 2002; van Montfrans, et al. 2002). Inhibition of p38 MAPK can powerfully reduce the production of pro-inflammatory cytokines and reduce intestinal inflammation (Feng and Li 2011). Macrophages are considered as the gatekeepers of intestinal immune homeostasis and their dysregulation leads to the chronic inflammation in IBD (de Souza and Fiocchi 2016). In our study, we found that macrophage-derived knockout of p38 α improved experimental colitis by reducing the secretion of pro-inflammatory cytokines TNF- α , IL-1 β and IL-6.

Intestinal barrier composed of mucous layer and epithelial cell layer can protect the intestinal mucosa from the attack of pathogens and antigens (Van der Sluis, et al. 2006). Previous study showed that muc-2

deficiency in mice spontaneously induces the colitis (Van der Sluis, et al. 2006). In addition, IECs are interconnected by tight junctions (TJs), which regulate paracellular permeability. The members of claudin family are the principal constituents of the TJs, of which claudin-2 favors immune-mediated colitis and targeting claudin-2 relieved its progression. However, our results indicated that macrophage-derived knockout of p38 α increased the enrichment of muc-2 and claudin-2 in experimental colitis, indicating that macrophage-derived knockout of p38 α might improve experimental colitis by promoting mucosal homeostasis.

MAPKs are involved in multiple physiological processes, including cell growth, metabolism, differentiation and cell death (Huang, et al. 2009; Roux and Blenis 2004; Zhang and Liu 2002). Activation of p38 MAPK suppresses the proliferation of corneal endothelial cells, whereas the p38 MAPK inhibitor counteracts this effect (Nakahara, et al. 2018). P38 α deficiency in neonatal muscle regulates cellular hyperproliferation and maturation (Perdiguero, et al. 2007). Neonatal mice lacking P38 α in cardiac muscle exhibit an increase in DNA synthesis and mitosis in cardiomyocytes (Engel, et al. 2005). Herein, we investigated the effects of macrophage-derived p38 α on colon cell proliferation and found that Ki-67 proliferation levels were markedly increased in experimental colitis in p38 $\alpha^{\Delta M\phi}$ group as compared with the WT group. In addition, increasing data indicate the pro-apoptotic role of p38 MAPKs in multiple cell types (De Zutter and Davis 2001; Ghatan, et al. 2000). We found that macrophage-derived knockout of p38 α reduced cell apoptosis of IECs in DSS-induced colitis. Our results suggested that macrophage-derived knockout of p38 α might improve experimental colitis by regulating cell proliferation and apoptosis of IECs.

It has been widely accepted that the pathogenesis of IBD is associated with the imbalance of intestinal mucosal immune responses to environmental factors in genetically-predisposed individuals (Pizarro, et al.). Innate and adaptive immune responses cause inflammatory lesions in IBD (Geremia, et al. 2013). Of note, p38 MAPK acts a prominent role in innate and adaptive immune responses (Su, et al. 1994). Our results identified that immune process-related factors including Lgals9, Rtp4, Ddx60, Nlrp1b, Hsh2d, Oas2 and Oas3 were enriched in p38 $\alpha^{\Delta M\phi}$ group as compared with the WT group. Lgals9 is a β -galactoside-binding lectin that acts in physiological and pathological conditions, such as immune response (John and Mishra 2016). It induces T cell apoptosis (Kashio, et al. 2003; Zhu, et al. 2005) and dendritic cell (DC) activation (Li, et al. 2011). Lgals9^{-/-} mice harbors DSS-induced intestinal injury (Robinson, et al. 2020), (Kim, et al. 2010). RTP4 controls IFN-I response by affecting TBK1 and IRF3 phosphorylation (He, et al. 2020). DDX60 acts as a ligand-specific sentinel activated by RIG-1 to participate in innate immune response (Miyashita, et al. 2011; Oliver, et al. 2015). Nalp1b, a member of (NOD)-like receptors family recognizes pathogen-associated molecular patterns, such as lipopolysaccharide (Nour, et al. 2009). HSH2D is a weighty signal molecule that involves in the activation of T cells (A, et al. 2001), leading to tumor malignancy and drug resistance in Tcell acute lymphoblastic leukaemia (Wang and Xiong 2020). The OASs are a family of IFN- and virus-induced proteins, consisting of OAS1, OAS2, OAS3, OASL (Liao, et al. 2020). OAS2 and OAS3 have antiviral activity (Li, et al. 2016; Liao, et al. 2020) and OAS3 negatively regulates chemokines and interferon-responsive factors in macrophages (Wook-Bin, et al. 2018). Our

results further validated that Lgals9, Rtp4, Ddx60, Nlrp1b, Hsh2d, Oas2 and Oas3 were upregulated in p38 α ^{ΔM ϕ} group as compared with the WT group, suggesting that macrophage-derived knockout of p38 α might improve experimental colitis by regulating immune process.

Pharmacological inhibition of p38 MAPK has been used to treat various inflammatory diseases, such as rheumatoid arthritis, Alzheimer's disease and IBD (Waetzig, et al. 2002). SB203580 as a p38 MAPK inhibitor reduces the production of IFN- γ and IL-12p70 in colitis (ten Hove, et al. 2002). Herein, our results suggested that p38 inhibitors could significantly ameliorate experimental colitis. This may provide a potential theoretical basis for treatment of UC.

Conclusion

Taken together, we unveil a novel role of macrophage-derived p38 α in colitis and found that macrophage-derived p38 α facilitates the pathogenesis of colitis by reducing inflammation response, protecting mucous barrier and regulating cell proliferation and immune process. Our work may provide a new approach for colitis treatment.

Declarations

Ethics approval and consent to participate

All experiments involving animals were conducted according to the ethical policies and procedures approved by the ethics committee of Shanghai Sixth People's Hospital (2020-0015). All subjects provided written informed consent and the study protocol was approved by the ethics committee of Shanghai Sixth People's Hospital. (2020-231).

Consent for publication

All the authors agreed to publish articles in the journal.

Availability of data and material

The data underlying this article will be shared on reasonable request to the corresponding author.

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Not applicable.

Authors' contributions

JZ and JSZ supervised and designed the study and WC drafted the manuscript. WC, HF and RL performed the experiments. WC, XC and YY conducted the statistical analysis. WC wrote the paper and JZ revised the paper. All authors read and approved the final manuscript.

Competing interests

All authors declare that they have no conflicts of interest.

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Tables

Table 1. List of primers of the genes.

Gene	Forward primer	Reverse primer
GAPDH	CCTCGTCCCGTAGACAAAATG	TGAGGTCAATGAAGGGGTCGT
Lgals9	CTCTTCAGTGCCCAGTCTCCA	CTGTCCGTTCTGCTTCGTGT
Rtp4	GAATGCTGTTCCCCGATGAC	GGAACACTGGAACCTGCCAAG
Ddx60	CTACTAGCTCCTCATCGCCAA	CTCCCAGATTTCCGCTCCA
Nlrp1b	AATCCTATGAGGCAGTGCGG	GCCAGGCTCCTATCAATGTCA
Hsh2d	ACACCCTTTCTACAAAGCCC	CTCCCCGAATGGCCGAATAG
Oas2	GAAGAAGCGAAGGAGTGGCT	GGGGGTCTGCATTACCTAGAC
Oas3	CCTGAGTTCACAGCAGCCGTA	CCCTTGGCAATCCTTATCACT
mucin-1	AAGTTCAGGTCAGGCTCCGT	GGGAGGGAACTGCATCTCATT
mucin-2	AGAAGCCAGATCCCGAAACCA	TCTCGGCAGTCAGACGCAAA
ZO-1	CAAAGCCCACCAAGGTCACAC	TTCAGTAAGGTAGCCCGCTCA
occludin	TAAAAGGGCTCCCACGAAGG	GGATATTCCCTGACCCAGTCC
claudin-1	ATTTCAAGTCTGGCGACATTAG	ACAGGAGCAGGAAAGTAGGACA
claudin-2	AGTGCGATATCTACAGTACCCTT	ATAATACAAGCCAGCGAGGACA

Figures

Figure 1

Macrophage p38α is upregulated in human colitis colon tissues. (A) Representative immunohistochemistry images of p-p38α in colitis and normal human colon tissues. (B) p-p38α protein expression in colitis (n=10) and normal human liver tissues (n=5). (C) Representative FISH images of CD68 and p38α in colitis and normal human colon tissues. (D) Analysis of the association between CD68 and p38α in human colitis colon tissues. (E) The expression of CD68, p38α and CD68-p38α in colitis (n=10) and normal human liver tissues (n=5). *P < 0.05

Figure 2

Macrophage p38α protects against DSS-induced colitis. (A) Body weight changes of WT and p38α^{ΔMφ} mice were monitored daily. (B) DAI of WT and p38α^{ΔMφ} mice were monitored daily. (C) Gross morphology images of colons of WT and p38α^{ΔMφ} mice. (D) Colon lengths of WT and p38α^{ΔMφ} mice. (E) Representative H&E staining of distal colon sections of WT and p38α^{ΔMφ} mice. (F) Histology scores of WT and p38α^{ΔMφ} mice. Data are showed as means ± SEM. *P < 0.05 and **P < 0.01.

Figure 3

Macrophage p38 α deletion inhibits pro-inflammatory cytokine production in DSS induced colitis. (A-C) The expression levels of TNF - α , IL-6 and IL-1 β in colon tissues were analyzed by IHC analysis. *P <0.05, **P <0.01, ***P <0.001.

Figure 4

Macrophage p38 α deficiency induces the secretion of muc-2 and claudin-2 (A) The relative mRNA expression of muc-2 and claudin-2 in colon tissues of DSS-treated WT and p38 α Δ M ϕ mice were analyzed by qRT-PCR. (B) Representative IHC images of muc-2 and claudin-2 in WT and p38 α Δ M ϕ mice. (C) Western blot of claudin-2 protein levels in colonic tissues of DSS-treated WT and p38 α Δ M ϕ mice. (D) Representative IF images of muc-2 and claudin-2 in WT and p38 α Δ M ϕ mice. Data are showed as means $\pm$ SEM. * $P < 0.05$ and ** $P < 0.01$.

Figure 5

Macrophage p38 α deficiency affects the proliferation and apoptosis of IECs and thus inhibits the course of colitis. (A) Ki-67 expression in WT and p38 $\alpha^{\Delta M\phi}$ mice was detected by immunohistochemistry. (B) The apoptosis of IECs was detected by TUNEL. Data are showed as means $\pm$ SEM. * $P < 0.05$ and *** $P < 0.001$.

Figure 6

Macrophage p38 α deficiency enhances immune system process during colitis (A) Heatmap of the differentially expressed genes between WT and p38 $\alpha^{\Delta M\phi}$ mice groups after DSS treatment. (B) Volcano plotting of the differentially expressed genes between WT and p38 $\alpha^{\Delta M\phi}$ mice groups after DSS treatment. (C-D) Go analysis was performed for genes with significant difference in expression. (E) The major genes that related to immune system process. (F) The mRNA expression of Lgals9, Rtp4, Ddx60,

Nlrp1b, Hsh2d, Oas2 and Oas3 in colonic tissues of WT and p38 α Δ M μ mice on day 7 after DSS treatment were determined by real-time PCR. (G) The protein expression of Oas2 and Oas3 in colonic tissues of WT and p38 α Δ M μ mice were determined by immunohistochemistry. Data are showed as means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Figure 7

The administration of p38 MAPK inhibitor restricts colitis in mice (A) Body weights of the four groups were monitored daily. (B) DAI scores of the four groups were recorded in each mice. (C) Colon lengths of each mice were calculated. (D) Representative pictures of colons from the four groups. (E) Representative H&E staining of distal colon sections of the four groups. (F) Histological scores of colon tissues were calculated. Data are showed as means $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$.

Figure 8

It has been reported that macrophage-derived p38α promotes liver injury and steatohepatitis progression. Herein, we estimated the role of macrophage-derived p38α in DSS-induced experimental colitis. We found that the expression of macrophage-derived p38α was elevated in colitis tissues, and macrophage-specific knockout of p38α improved the clinical symptoms, DAI and histopathological scoring

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