

STAT3-mediated T_H17 cell Differentiation was Related to the Carcinogenesis of Colitis Related Cancer

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Abstract

Background: Inflammation often induces regeneration to repair the tissue damage. However, chronic inflammation can transform temporary hyperplasia into a fertile ground for tumorigenesis. Here, we demonstrate that the miR-124 acts as a safeguard to inhibit the pro-inflammatory production and reparative regeneration.

Methods: The expression levels of miR-124 and IL-17, IFN- γ were analyzed by quantitative real-time polymerase chain reaction (qRT-PCR). T_H17 or T_H1 cells were detected by flow cytometer, respectively. the binding of STAT3 to the promoter region of IL-17 gene was analyzed by Chip assay. miR-124 binding to the 3'UTR of STAT3 gene was detected by reported plasmid construction and luciferase assay. Furthermore, DSS-induced colitis mice model and T cell transfer model were used to confirm the function of miR-124 *in vivo*. The related gene expression was analyzed by ELISA and western blot experiments.

Results: The results indicated that miR-124a deficiency leads to colon tumorigenesis after *Citrobacter rodentium* infection and AOM/DSS induced colon cancer murine model. In molecular mechanism, miR-124 targets STAT3 to suppress T helper 17 (T_H17) cell differentiation and expansion, and keep T_H17 polarization in colonic microenvironment.

Conclusions: Our study highlights highlight the potential role of miR-124 in the control of immune responses and pathogenesis of inflammatory diseases.

Background

CRC progression is a process that involves interactions between the tumor and the host cellular immunity in the tumor microenvironment. Neoplastic cells secrete pro-inflammatory mediators and immune cells produce cytokines which all lead to tumor development. Studies have shown that the number of Th17 cells are significantly higher in CRC tissues [1, 2]. Th17 cells induce immune suppressive mediators such as TGF- β , CXCR3, CC chemokine receptor 6 (CCR6) and IL-6 and also suppress CD8⁺ T cells which have anti-tumor activity. Moreover, it has been shown that the number of IFN- γ producing CD8⁺ T cells is increased in the IL-17 deficient mice [1, 3]. IL-17 is produced by Th17 cells and it is an important cytokine in various immune responses such as type 2 immune response. In pro-inflammatory responses, IL-17 plays an important role in activation and recruitment of neutrophils. Neutrophils are the main sources of cytokines related to Th2-type immune response that induce negative feedback, suppress neutrophil, decrease tissue destruction, and also induce IL-17 production [4]. Published data demonstrated that the level of IL-17 was significantly higher in most of CRC tissues. The up-regulation of IL-17 begins from adenoma stage and its level is higher at the cancer stage but it is not associated with TNM parameters of the tumor [5, 6]. Therefore, IL-17 is involved in CRC tumorigenesis through several pathways and underlying molecular mechanism.

MicroRNAs (miRs) are small non-coding RNA oligonucleotides that can regulate the expression of a large number of genes and have been involved in different human diseases [7]. MiRs are centrally involved in the pathogenesis of different human inflammatory diseases, including IBD. Many published documents identified that miRs are essential regulators of Toll-like receptor signaling, which is important to trigger the intestinal inflammation. For example, miR-146b induced by IL-10-IL-10R signaling regulated the Toll-like receptor 4 (TLR4) by negative feedback in human monocytes [8], and miR-146b deficient mice were easier to develop colitis by targeting IRF5 [9], which was regarded as a regulator of TLRs in LPS-driven TLR signaling. Moreover, many evidences have indicated that inflammation may contribute to carcinogenesis and IBD can increase the risk of colorectal cancer. However, it is still elusive to elucidate the details of the relationships between IBD and CRC [10]. Josse et al summarized the recognized molecular mediators which linked inflammation to colorectal cancer, including cytokines, growth factors, Toll-like receptors, PI3K/MAPK signaling, NF- κ B/STAT3 signaling, Wnt signaling way [11]. In addition, more recent studies have indicated that miRNAs can target above signaling molecules and connect inflammation to cancer development. Yuan et al reviewed and listed that miRNAs were involved in inflammation to cancer. For example, miR-126 could directly target the CXCR4 or PI3K/AKT signaling pathway on tumor suppression [12, 13]. Among those, miR-124 is deregulated specifically in pediatric patients with active UC, leading to increased levels of STAT3 expression and the transcriptional activation of its downstream targets. Moreover, in active pediatric UC the miR124/STAT3 pathway is epigenetically regulated, suggesting the involvement of epigenetic-transcription regulatory circuits in the pathogenesis of pediatric UC [14]. But, it is still unclear whether miR-124 can mediate the colitis related colon cancer progression.

In this study, we observed that miR-124 could inhibit the Th17 cell proliferation and was down-regulated in Th17 cell differentiation. The miR-124 mimic would retain in the inflamed area and efficiently inhibited the Th17 polarization, causing the phenotype transition into Treg cells, which next inhibited the inflammatory response and promoted the mucosal regeneration, and finally decreased the colitis-related colonic cancer development. Therefore, we expect targeting miR-124 would offer a novel therapeutic strategy for colitis-related colonic cancer.

Methods

Mice

C57BL/6J and Rag1^{-/-} mice were obtained from Model Animal Research Center of Nanjing University and maintained in the barrier facility at Guangzhou Medical University. To induce MC38 tumor-bearing mice model, 5 × 10⁵ MC38 cells were injected subcutaneously into mice. The animal study protocols were approved by the Institutional Animal Care and Use Committee of Sun Yat-sen University Cancer Center.

Reagents and antibodies

MiR-124 mimic and inhibitor were purchased from GenePharma. The following Flow cytometry antibodies were purchased from BD Biosciences (USA), and conjugated to FITC, PE, PE-Cy5, PE-Cy7, PerCP-Cy5.5, PerCP-eFluor 710, eFluor450 or APC: CD45RB (C363.16A), CD4 (L3T4), CD25 (PC61.5), IL-17 (TC11-18H10), IFN- γ (XMG1.2), FOXP3 (EJK-16S) and isotype controls. Antibodies for ROR γ (B2D), Gr1 (RB6-8C5) and IL-4 (11B11) were purchased from eBioscience.

FITC Annexin V Apoptosis DetectionKit I (2293683) was purchased from BD Pharmingen. Anti-ROR γ t, anti-T-bet (MBL), anti-STAT3, anti-pSTAT3 (CellSignaling), and anti- β -actin (Sigma) antibodies for western blotting were used according to the manufacturers' instructions. Secondary antibodies were from Santa Cruz Biotechnology, Inc.

CD4⁺ T cell preparation and differentiation in vitro

Naive CD4⁺ T cells (CD62L+CD44^{lo}) were prepared by fluorescence-activated cell sorting from spleens and lymph nodes of C57BL/6 mice. The sorted cells were primed for 96 hrs with anti-CD3 (1 μ g/ml; 145-2C11; BDBiosciences) and soluble anti-CD28 (2 μ g/ml; 37.51; BDBiosciences). Cells stimulated under neutral conditions were defined as TH0 cells. Cells were stimulated to differentiate into TH1 cells by supplementation with IL-12 plus anti-IL-4 or into TH2 cells by supplementation with IL-4 and anti-IFN- γ . For TH17 cell differentiation, cells were stimulated with transforming growth factor- β 1 (5 ng/ml), IL-6 (20 ng/ml) and IL-23 (10 ng/ml; all from R&D Systems) and into Treg cells by supplementation with transforming growth factor- β 1 (15 ng/ml).

Intracellular staining and flow cytometer

For T cells, cells were stimulated with PMA and ionomycin for 5 hrs in the presence of brefeldin A prior to intracellular staining. Cells were fixed with IC Fixation Buffer (BD Bioscience), incubated with permeabilization buffer, and stained with antibodies. For macrophages, bone marrow derived macrophages were activated with LPS (200 ng/ml) plus IFN- γ (10 ng/ml) overnight and brefeldin A was added to the culture for 5 hrs prior to intracellular staining. Flow cytometry was performed on a FACS Calibur (BD Biosciences).

Cytokine ELISA

Supernatants from cell cultures were collected after activation under various conditions and secreted cytokines in the supernatants were measured by ELISA kits with purified coating and biotinylated detection antibodies: anti-IL-17, anti-IFN- γ (R & D systems), anti-IL-9, anti-IL-22, anti-IL-1 β (e Bioscience) and anti-IL-4 (BDBioscience).

RNA isolation and quantitative real-time RT-PCR (qPCR)

Total RNA was extracted using an RNeasy plus kit (QIAGEN, Valencia, CA) and cDNA was generated with an oligo (dT) primer and the Superscript II system (Invitrogen, USA) followed by analysis using iCycler PCR with SYBR Green PCR master Mix (Applied Biosystems). Results were normalized based on the expression of ubiquitin. The sequences of primers are shown in Supplementary Table 1.

T cell-transfer colitis

T cell transfer colitis was performed as previously described. Briefly, purified CD4⁺CD45RB^{hi} T cells from WT mice were injected intraperitoneally into Rag1^{-/-} recipients (5 x 10⁵ cells per mouse in 200 μ l sterile PBS per injection) [15]. Mice were weighed every week throughout the course of experiments. The degree of inflammation in the epithelium, submucosa and submucularis propria was scored separately as described previously [9].

Statistical analysis

The results are shown as means \pm SD and statistical analysis was performed using Student's t-Test. Where more than two groups were compared, one way-ANOVA with Bonferroni's correction were performed. P < 0.05 was considered statistically significant.

Results

MiR-124 inhibited the T_H17 cell polarization

To investigate the effects of miR-124 on adaptive immune cell function, we first focused on T helper cells. Naïve CD4⁺ T cells from C57BL/6 mice were primed in vitro for 3 days under T_H0, T_H17, Treg and T_H1 conditions and miR-124 expression was evaluated by qPCR. We found that miR-124 expression was obviously decreased in T_H17 cell rather than TH1 (Figure 1A). qPCR and ELISA experiments showed that miR-124 mimic significantly suppressed expression of T_H17 or T_H1-associated genes including IL-17, IFN- γ (Figure 1B,C,D,E). We next detect whether the T_H17 and T_H1 cell differentiation was affected in the presence of miR-124 mimic. These observations correlated with reduced IL-17 and IFN- γ production by T_H17 or T_H1 cells treated with miR-124 mimic as determined by flow cytometer (Figure 1F). The results showed that the frequency of IL-17- and IFN- γ -producing cells decreased following miR-124 mimic treatment. To rule out the possibility that the reduced TH17 and TH1 cell differentiation was due to abnormal cell apoptosis caused by miR-124, we isolated and analyzed CD4⁺ T cells from spleens as well as lymph nodes of C57BL/6 mice by Annexin V and PI staining or CFSE staining. The results showed that miR-124 mimic administration did not increase the T cell apoptosis but affected the CD4⁺ cell proliferation (figure G,H).

MiR-124 alters DNA binding activity in T_H17 cells

Furthermore, the evidence prompted us to probe for the molecular basis for how miR-124 modulated T_H17 cell differentiation. Since many studies have shown that several transcription factors including ROR γ t, STAT3, and AHR are important for T_H17 cell differentiation, we hypothesized that miR-124 might affect the expression of these transcription factors. To address this, naïve CD4⁺ T cells from C57BL/6 mice were primed in vitro for 3 days under T_H0 or T_H17 polarizing conditions. IL-17 mRNA was detected in TH17 polarization in the presence of miR-124 mimic and the results showed that IL-17 was inhibited by miR-124 mimic (Figure 2A). However, the levels of ROR γ t and AHR protein were comparable in the presence of miR-124 compared with control (Figure 2B), but STAT3

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IL-17 gene was significantly reduced (Figure 2D). The data suggested that miR-124 could suppress the STAT3 expression and subsequently affect the IL-17 expression in Th17 cell differentiation condition.

STAT3 is the target of miR-124

We further investigated how miR-124 affect the STAT3 in Th17 cell differentiation. The previous document and bioinformatics analysis revealed that miR-124 could bind the 3'UTR of STAT3 gene, and inhibit the protein translation. To verify that miR-124 really targets STAT3, we first detected that STAT3 was affected in the presence of miR-124 mimic, and the results suggested that miR-124 mimic decreased the STAT3 expression in EL4 cell line (Figure 3A). Next, we constructed the luciferase reporter plasmids of the STAT3 3'UTR of mouse (including WT and miR-124 bind site mutant plasmids). The plasmids were then respectively co-transfected with the miR-124 mimic to E14 or HEK293T cells. Interestingly, transfection of miR-124 mimic significantly decreased the luciferase activity in group transfected with STAT3 plasmid; however, miR-124 mimic had no effect on luciferase activity in group transfected with STAT3 mutant plasmid (Figure 3B,C,D). RNA immunoprecipitation (RIP) experiments demonstrated that the miR-124 and STAT3 mRNA were in the same miRNA-induced silencing complex (miRISC) (Figure 3E). Taken together, the results suggest miR-124 modulates TH17 differentiation by targeting STAT3.

MiR-124 weaken the colitis in CD4 positive cell transfer model

Previous reports have indicated a correlation between T_H17 activity and IBD pathogenesis. To further assess the effects of miR-124 on T_H17 cell development in vivo, we performed adoptive T cell transfer colitis experiments using $CD4^+CD45Rb^{hi}$ cells from C57BL/6 mice to induce colitis in $Rag1^{-/-}$ mice. Mice in the treatment group received low dose miR-124 twice a week for 8 weeks while the control group was treated with PBS. While the $Rag1^{-/-}$ mice reconstituted with naïve $CD4^+$ T cells lost weight continuously, treatment with miR-124 significantly improved their condition (Figure 4A,B). Parallel histologic studies of colonic sections from $Rag1^{-/-}$ mice treated with miR-124 revealed fewer inflammatory cell infiltration and significantly lower pathological scores compared to mice treated with PBS (Figure 4C). And, miR-124 expression also was confirmed in the treatment of miR-124 mimic (Figure 4D). In addition, mice treated with miR-124 had significantly lower percentages of IL-17 cells and lower expression of T_H17 and T_H1 signature gene expression than PBS treated mice (Figure 4E,F).

MiR-124 suppressed the development of colitis associated carcinoma by inhibiting the T_H17 differentiation

Ulcerative colitis (UC) is a subcategory of inflammatory bowel disease (IBD) with high risk of colorectal cancer. 2 key pathophysiological features of this disease are dysregulation of immune system and impaired mucosal repair. As previously reported, T_H17 cell differentiation was closely related to CAC occurrence. After the third cycle of CAC regimen suspended, we had successfully created a colitis associated carcinoma model and observed palpable tumors near the rectum of mice. Interestingly, miR-124 mimic treatment group developed significantly smaller tumor numbers and tumor areas than control group (Figure 5B). In addition, histological assessments showed that the colonic mucosa in miR-124 mimic treatment mice presented with low-grade dysplasia, but the tumors of control group were usually identified as high-grade dysplasia (Figure 5C). Strikingly, in the process of acute mucosal injury, miR-124 mimic treated mice exhibited better epithelium structures concomitant with down-regulation of IL-17. While during the CAC, IL-17 expression in colonic mucosa of miR-124 mimic treated mice was lower than that in control group (Figure 5D,E), suggesting that in the background of AOM induced missense mutations, chronic inflammation impaired the epithelial microenvironment and the T_H17 cells function, resulting in development of inflammation induced cancer, and T_H17 cells recruited around the neoplastic epithelial cells in regeneration, and the concomitant alteration was also observed in STAT3. Therefore, miR-124 suppressed the development of CAC by attenuated the "the second hit" of cancer.

MiR-124 depressed the colon carcinogenesis in *C. rodentium* infection colitis colon cancer murine model

Microbial dysbiosis causes chronic inflammation associated with CRC. *C. Rodentium* is a mouse mucosal pathogen that shares pathogenic mechanisms and 67% of its genes with enteropathogenic Escherichia coli (EPEC) and enterohaemorrhagic E. coli (EHEC), which are two clinically important human gastrointestinal pathogens. *C. Rodentium* has been used as a model to study mucosal immunology, including intestinal inflammatory responses during bacteria-induced colitis and colon tumorigenesis. *C. rodentium* infection increases the number of colonic adenomas in Apc^{Min} mice but does not cause adenoma formation in wild-type mice.

After *C. Rodentium* (2×10^9 CFU) infection, wild-type mice developed diarrhea and weight loss within 2 weeks, and were then divided into two groups: miR control and miR-124 mimic. Until 6 months, all mice were sacrificed and performed the histologic staining analysis. Microscopic sections from WT control mice were free of dysplastic and neoplastic changes at six-month time points following infection. In miR-124 mimic treatment mice, less dysplasia or early neoplasia was present at this time point, whereas 16/20 (wt control) vs 5/20 (miR-124 mimic) mice had microscopic changes ranging from dysplasia to adenocarcinoma (Figure 6 A,B,C). We further isolated $CD4^+$ T cells from *C. Rodentium*-infected colon cancer tissue and analyzed the relative abundance of T_H17 subpopulations according to their associated expression of IL-17. IL-17 was significantly decreased after miR-124 treatment (Figure 6 D,E). So, miR-124 mimic treatment depressed the T_H17 cell differentiation in the colon post *C. Rodentium* infection.

Discussion

Ulcerative colitis (UC) is a subcategory of inflammatory bowel disease (IBD) with high risk of colorectal cancer, which included two key pathophysiological features: dysregulation of immune system and impaired mucosal repair [16]. Limited therapies available at present remains UC and colitis-related colon cancer a challenging problem for the clinician. In our study, we confirmed the miR-124 was a key regulator for T_H17 cell differentiation in UC and CAC, and performed the miR-124 mimic to effectively inhibit the inflammation and colon cancer occurrence in murine colitis model. Thus miR-124 could inhibit T_H17 cell differentiation and pro-inflammatory cytokines induction, thereby promoting the mucosal repair and suppressing the development of colitis

associated carcinoma. We concluded that miR-124 is a critical regulator in control of intestinal immune function and epithelial regeneration, and targeting miR-124 can be selected as a “smart” therapeutic strategy for UC and colitis-related colon cancer.

After tumor formation in colon, immune system reacts against neoplastic cells. Immune responses include immune cells proliferation, phenotype alteration, synthesis and release of cytokine [17], such as interleukin-17 (IL-17). IL-17 is a pro-inflammatory cytokine, which is associated with cancer progression [18, 19]. The main source of IL-17 is a subpopulation of CD4⁺ T cells known as T-helper17 (Th17) cells. Tumor infiltrating Th17 cells were found in many types of cancers [19, 20]. In colon cancer, published document showed that Th17 was involved in colitis and colitis related cancer [3, 21]; Th17 cells was related to tumor angiogenesis [22], and also could mediate the activity of CTLs in colon cancer development [3]. Although physiologic levels of inflammation were protective, excessive inflammation was deleterious and was at the basis of inflammatory bowel disease (IBD) and inflammation-promoted colorectal cancer. Production of cytokine together with that of matrix-degrading enzymes, growth factors, and reactive oxygen species promote tumorigenesis by creating a microenvironment favoring intestinal epithelial cell proliferation, cell survival, and invasiveness. In our study, Th17 also was verified to be related with colon cancer development. But how to regulate the Th17 cell differentiation still was elusive.

Several agents are required for differentiation and stabilization, such as transforming growth factor beta (TGF- β), interleukin-6 (IL-6), IL-21, IL-23 and IL-1b. Also, retinoic orphan receptor- γ (ROR γ) and signal transducer and activator of transcription 3 (STAT3) are the transcription factors responsible for Th17 differentiation and stabilization. Gerogios et al demonstrated that miR-124 could promote the children UC and pathogenesis by regulating the expression and phosphorylation of STAT3, but special cell type was not involved [14]. The previous study also demonstrated that miR-124 depression was related to carcinogenesis, and development by targeting different gene [23, 24, 25]. These observations suggested that miR-124 played a key role in colitis and sporadic colon cancer. In our present study, we focused on the miR-124 function in Th17 cell and found that miR-124 could inhibit the polarization of Th17 cell and promote the transition of Th17 to treg in colitis and colitis related colon cancer by targeting stat3 gene. These results are consistent with downregulation of miR-124 developing intestinal failure with M1 macrophage phenotype by targeting stat3 and acetylcholinesterase (AChE). We believe that in the absence of miR-124 signaling cascade, the presence of intestinal commensal bacteria will drive intestinal CD4⁺ T helper cells toward Th17 cell polarization, resulting in a hyper-inflammatory response with associated tissue damage and pathogenesis.

Thus, our studies demonstrate that miR-124 expressed in Th17 cell modulates shaping of Th17 phenotype. We suggest a novel mechanism for the effect of miR-124 targeting STAT3 in the modulation of Th17 cell differentiation. Treatment with miR-124 mimic significantly suppressed Th17 cell activation by inhibiting the expression of STAT3 and IL-17 signature genes in vitro and ameliorated colitis and colon cancer development in vivo. Taken together, the results firmly establish miR-124 plays an important role in the modulation of Th17 cell activation and highlight the potential role of miR-124 in the control of immune responses and pathogenesis of inflammatory diseases.

Conclusions

Our study highlight the potential role of miR-124 in the control of immune responses and pathogenesis of inflammatory diseases. It is important that miR-124 mimic suppressed the TH17 differentiation during colitis process and delayed or inhibited the development of colitis-related cancer. miR-124 maybe could acted as biologic therapy point in colitis.

Abbreviations

CRC: Colitis Related Cancer

miR-124: microRNA-124

qPCR : quantitative real-time PCR

DSS: Dextran Sulfate sodium salt

AOM: azoxymethane

Declarations

Ethics approval and consent to participate

The animal study protocols were approved by the Institutional Animal Care and Use **ethical** Committee of Sun Yat-sen University Cancer Center.

Consent for publication

All authors reached the agreement for the publication

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

Conceptualization and methodology: SX and JW; Formal analysis and data collection: JW ,YG and KB; Writing—original draft preparation and Writing—review and editing: SX and JW; Approval of final manuscript: all authors. All authors read and approved the final manuscript.

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Figures

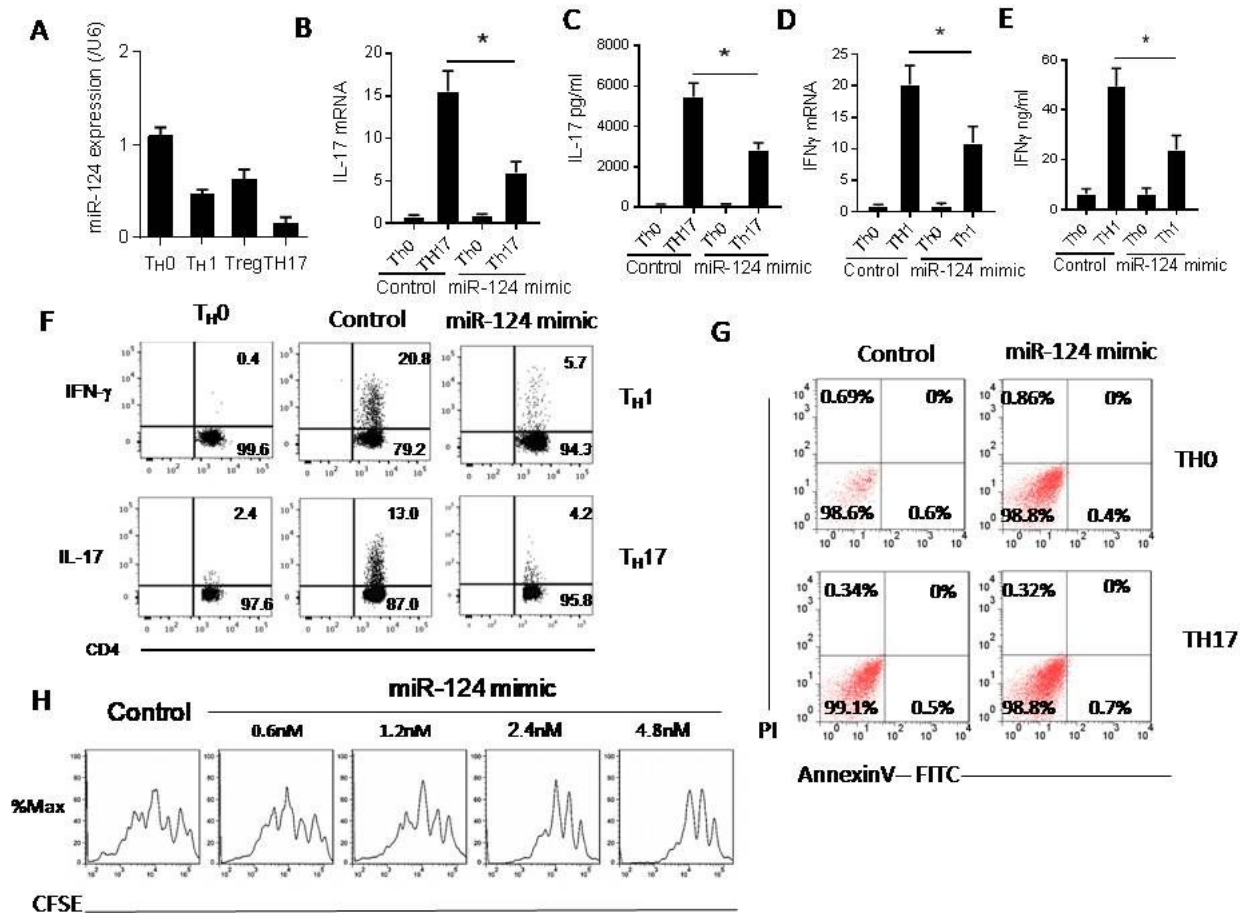


Figure 1

miR-124 selectively suppresses TH17 and TH1 cell differentiation. A. miR-124 expression in TH0, TH17, TH1 and treg polarizing conditions by qPCR assay. Naïve CD4⁺ T cells from C57BL/6 mice were differentiated under TH17 and TH1 polarizing conditions respectively in the presence of 4.8nmol miR-124 mimic for 3 days and analyzed through qPCR assay (B,C), ELISA (D,E), and flow cytometry (F). G. Naïve CD4⁺ T cells from C57BL/6 mice were differentiated under TH17 polarizing conditions respectively in the presence of 4.8nmol miR-124 mimic for 3 days and analyzed through flow cytometry. H. CD4⁺ cell proliferation was detected by CFSE. G. Naïve CD4⁺ T cells from C57BL/6 mice were differentiated under TH17 polarizing conditions respectively in the presence of 4.8nmol miR-124 mimic for 3 days, and stained by Annexin-V, analyzed through flow cytometry. *p < 0.05, **p < 0.01, ***p < 0.001 versus cells cultured, all tests were performed three times.

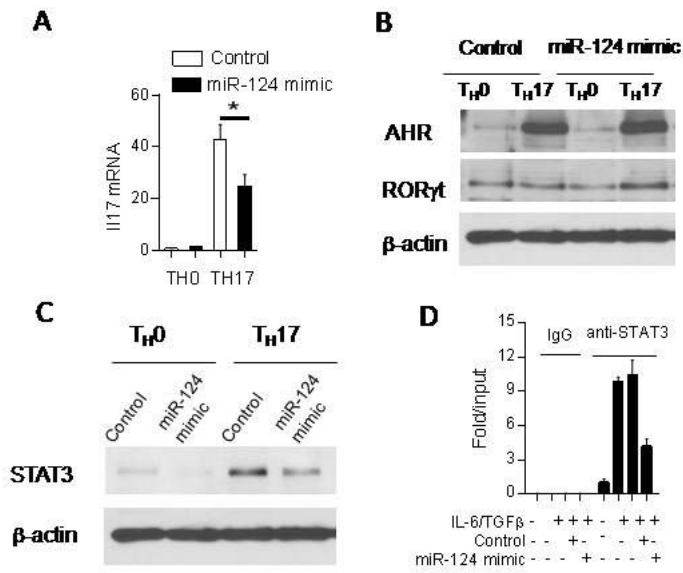


Figure 2

miR-124 suppresses TH17 activation by targeting STAT3. Naïve CD4⁺ T cells from C57BL/6 mice were differentiated under TH17 and TH1 polarizing conditions with IL-6 (10 ng/ml) and TGFβ (5 ng/ml) for 24 or 72 hrs in the presence of 4.8nmol miR-124 mimic. (A), miR-124 expression was detected by qPCR, U6 as control. (B,C).The whole cell lysates were prepared and western blotting was performed for the analysis of protein expression. b-actin expression serves as a control. (D) E14 cell was stimulated with CD3 and CD28 antibody and primed with IL-6 and TGFβ in the presence of miR-124 mimic, Chip assay was performed with anti-STAT3 antibody, IgG antibody as negative control. *, P<0.05; **, P<0.01. The results are representative of three independent experiments.

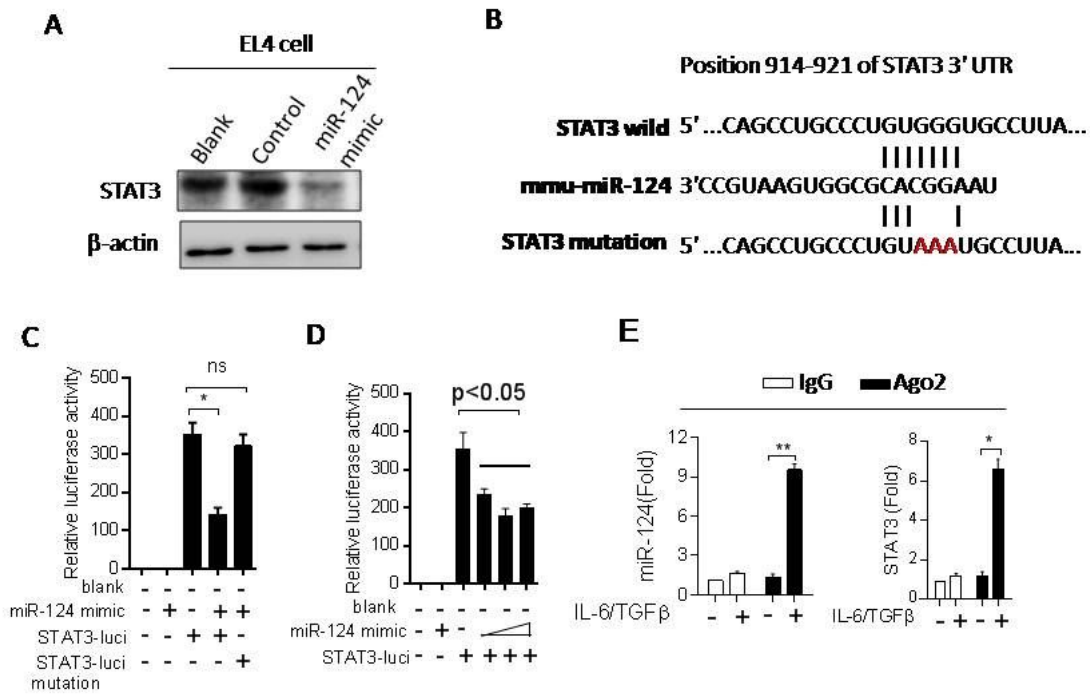


Figure 3

STAT3 is the target of miR-124 (A). EL4 cells were primed with IL-6 and TGF β in the presence of miR-124 mimic, STAT3 protein level was evaluated by western blotting. (B) Schematic representation of wild-type (wt) and mutant (mut) STAT3 3' UTR luciferase reporter construct of predicted miR-124 target. (C) EL4 cells were co-transfected with miR-124 mimic and STAT3-luciferase reporter plasmid after priming with IL-6 and TGF β . (D) 293 T cells were co-transfected with either WT or mutant STAT3 luciferase reporter plasmids together with the miR-124 mimic for 48 hrs. The cell lysates were prepared and luciferase activity was determined (Data represent mean \pm s.d.). (E) EL4 cells were primed with IL-6 and TGF β in the presence of miR-124 mimic for 24 hrs. Total RNA was extracted and immunoprecipitated with anti-Ago2 antibody. The immunoprecipitated RNA was purified and qPCR was performed for the analysis of miR-124 and STAT3 mRNA expression (Data represent mean \pm s.d.). The results are representative of three independent experiments (Data represent mean \pm s.d.). The results are representative of two independent experiments.

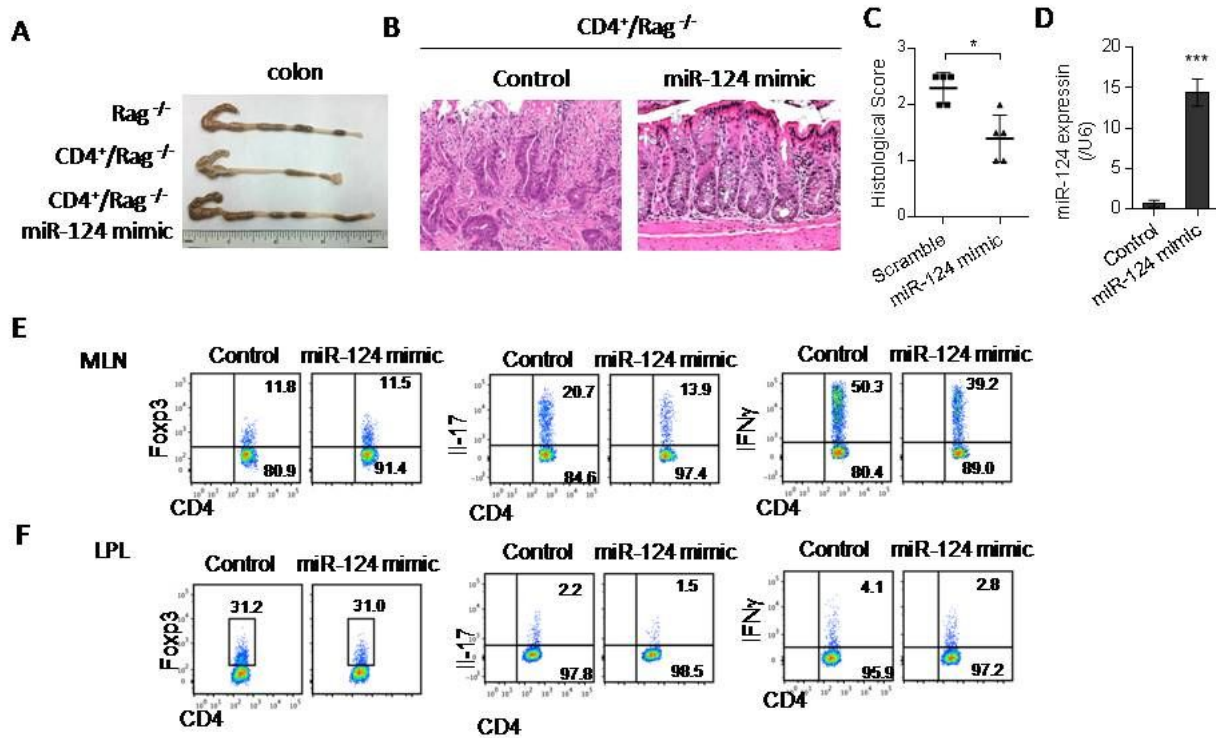


Figure 4

MiR-124 weakens colitis in CD4 positive cell transfer model in vivo. CD4⁺CD45Rb^{hi} T cells were purified from C57BL/6 mice and 5 × 10⁵ cells were injected (i.p.) into recipient Rag1^{-/-} mice. Mice were treated with control or miR-124 mimic (at 10 nm/mouse) every three days. (A), Morphology of intestines; (B), disease scores, *p < 0.05 versus recipients of control group (n = 5-6 mice per group), (C), sections of colons with colitis from Rag1^{-/-} mice (n = 5-6 mice in each group) 8 weeks after naïve T cell transfer as described above. Scale bar, 100 μ M. (D,E), The percentage of IL-17-producing cells from mesenteric lymph nodes and LPL of Rag1^{-/-} mice in control and miR-124 mimic treated group. **p < 0.01 versus recipients of control treated group.

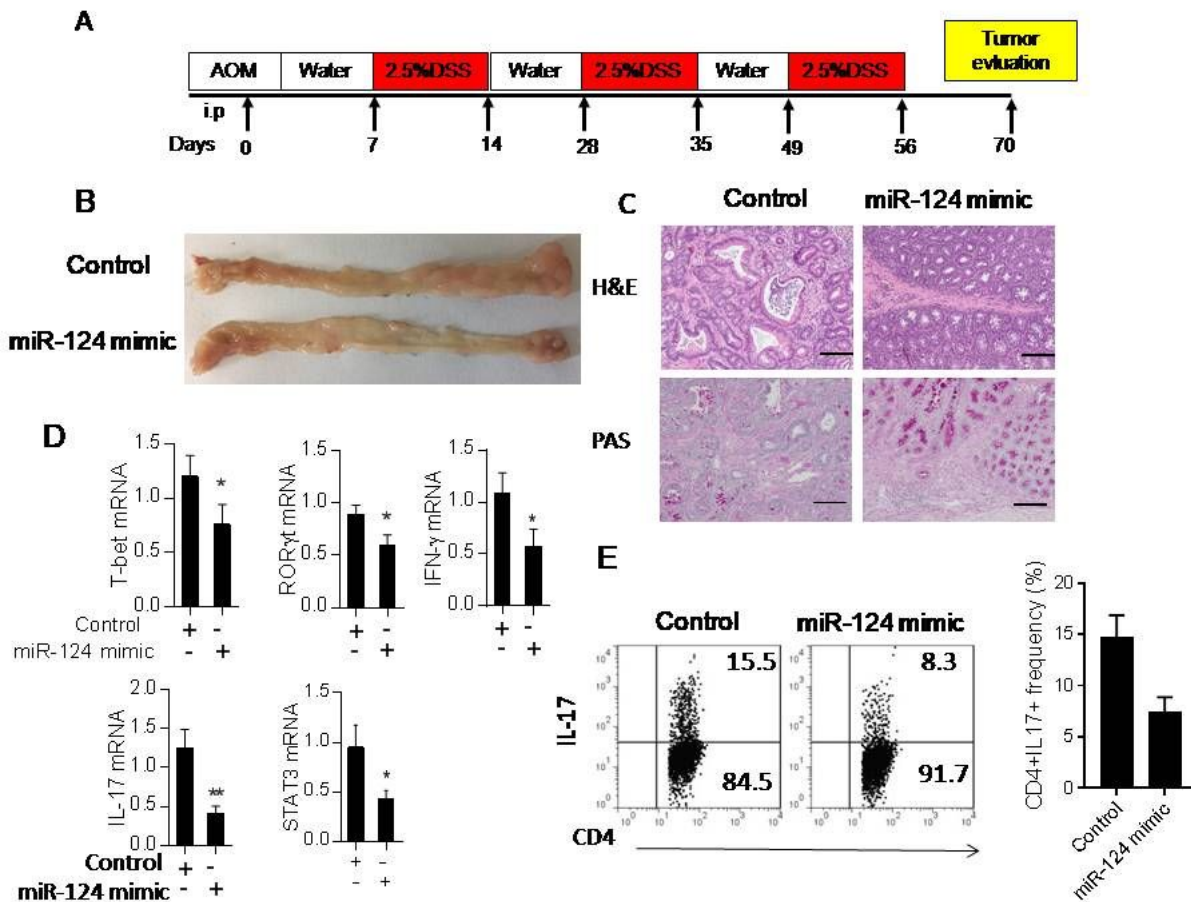


Figure 5

miR-124 suppressed the development of colitis associated carcinoma by inhibiting the TH17 differentiation. (A) Schematic of AOM/DSS administration. Mice were injected with 12.5 mg/kg AOM and subjected to three 7-day cycles of 2.5% DSS respectively in the presence of control or miR-124 mimic, (B,C) Morphology of intestines and representative H&E-stained sections and PAS after DSS or AOM/DSS treatment. Scale bar:100 μ m. (D). Total RNA was extracted and mRNA was detected by qPCR, b-actin as control, *, $P < 0.05$; **, $P < 0.01$. The results are representative of three independent experiments, (E). The percentage of IL-17-producing cells from mesenteric lymph nodes of mice in control and miR-124 mimic treated group. * $p < 0.05$ versus recipients of control treated group.

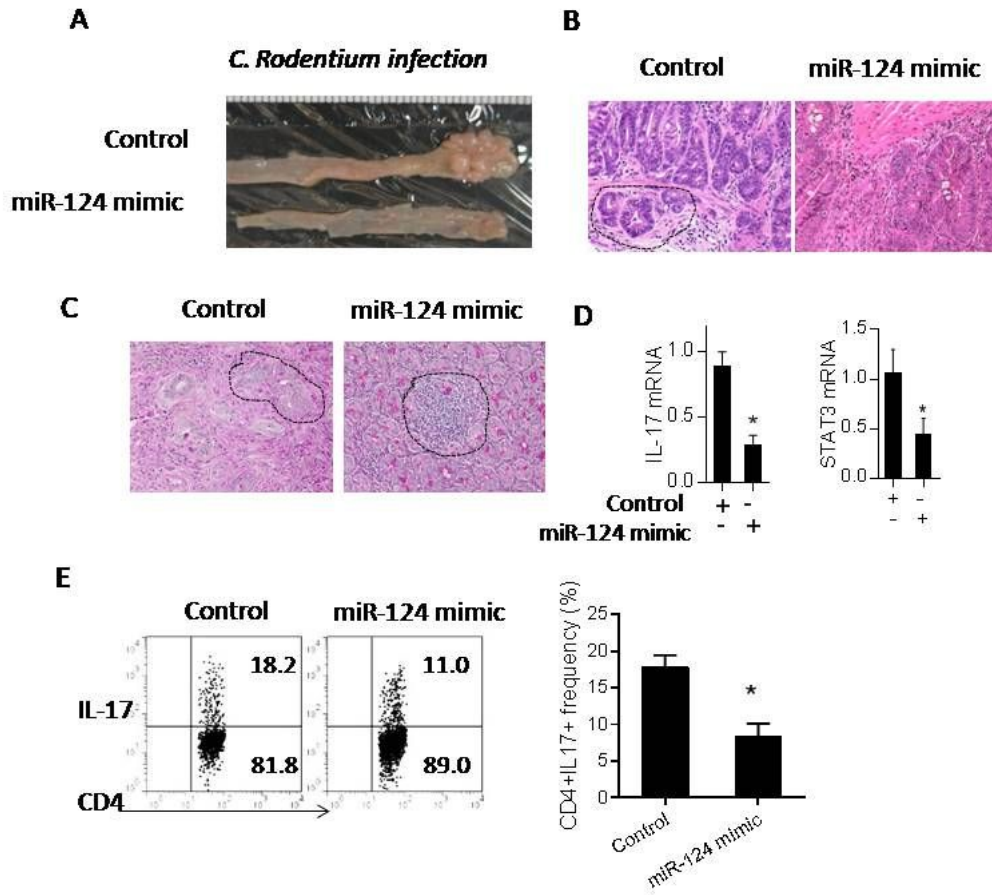


Figure 6

MiR-124 depressed the colon carcinogenesis in *C. rodentium* infection colitis related cancer murine model. 8-week-old wild-type mice (WT; n = 12) infected orally with 2×10^9 colony-forming units (CFU) of *C. rodentium* in the presence of control (n=6) or miR-124 mimic (n=6). (A). Morphology of intestines and representative H&E-stained sections and PAS after *C. rodentium* infection. Scale bar: 100 μ m. (D), Total RNA was extracted and mRNA was detected by qPCR, β -actin as control, *, $P < 0.05$; (E), the percentage of IL-17⁺-producing cells from mesenteric lymph nodes of mice in control and miR-124 mimic treated group. * $p < 0.05$ versus recipients of control treated group.