

# Role of microRNAs in the pathophysiology of ulcerative colitis

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## Supplementary Material

**Supplementary Table S1.** Role of UC-associated miRNAs in intestinal epithelial permeability.

miRNA	Expression in UC	Target	Function
miR-16	Elevated	CLDN2	The upregulation of miR-16 decreased claudin-2 expression and increased TEER in T84 cells[1].
miR-21	Elevated	Unspecified	The overexpression of miR-21 in Caco-2 cells impaired the integrity of the tight junctions and resulted in a decrease of TEER and an increase of the inulin permeability[2].
miR-29a/b	Elevated	CLDN1 NKRF	MiR-29 reduced the expression of claudin-1 and NKRF to increase permeability in intestinal epithelial cells[3].
miR-31	Elevated	ACVRL1	MiR-31-5p targets ACVRL1 responsible for colonocyte differentiation in human colonic epithelial cells and may thereby impair epithelial barrier integrity[4].
miR-125b	Elevated	CGN	The downregulation of miR-125b increased cingulin expression and decreased TEER in T84 cells[1].
miR-142	Elevated	CLDN1	The overexpression of miR-142-5p in thyrocytes reduced claudin-1 expression and increased the permeability of thyrocytes monolayer <i>in vitro</i> [5].
miR-155	Elevated	CLDN1	MiR-155 targeted CLDN1 and suppressed the invasive capacity of ovarian cancer-initiating cells in a Transwell migration assay[6].
miR-223	Elevated	CLDN8	IL-23-induced miR-223 targets CLDN8 and decreases TEER in colonic epithelial cells[7].
miR-200a/b/c	Reduced	OCLN CDH11	IL-1 $\beta$ -induced miR-200c-3p downregulated occludin expression in enterocytes and thereby increased epithelial permeability[8]. MiR-200c-3p directly regulates CDH11 expression[9].

ACVRL1, activin A receptor-like type 1; CDH11, cadherin-11; CGN, cingulin; CLDN1, claudin-1; CLDN2, claudin-2; CLDN8, claudin-8; OCLN, occludin; NKRF, nuclear factor- $\kappa$ B-repressing factor; TEER, transepithelial electrical resistance

**Supplementary Table S2.** Role of UC-associated miRNAs in innate immunity.

miRNA	Expression in UC	Target	Function
miR-16	Elevated	Pdcd4	MiR-16 drives macrophages towards the M1 phenotype[10]. MiR-16 directly targets Pdcd4 to suppress the activation of inflammatory macrophages in atherosclerosis[11].
miR-21	Elevated	Tlr4 PTEN PDCD4	LPS stimulation induces miR-21 expression in NR8383 alveolar macrophages, which in turn suppress the LPS-mediated induction of IL-1 $\beta$ , IL-6, and TNF- $\alpha$ by targeting Tlr4[12]. MiR-21 suppress LPS-mediated NF $\kappa$ B activation by targeting PTEN in human monocyte-derived macrophages, while it promotes IL-10 production by targeting PDCD4[13].
miR-125b	Elevated	Irf4	MiR-125b drives bone marrow-derived macrophages to adopt a pro-inflammatory phenotype at least partially by targeting Irf4 in mice[14].
miR-142	Elevated	IL6	LPS-induced miR-142-3p directly targets IL-6 in DCs[15].
miR-146a/b	Elevated	IRAK1 TRAF6 RIPK2 Notch1 INHBA	MiR-146a reduces the induction of TNF- $\alpha$ , IL-1 $\beta$ , IL-6, and MCP-1 by targeting IRAK1 and TRAF6 in mycobacteria-infected macrophages[16]. MiR-146a limits NOD2 signaling by targeting RIPK2 and suppresses the production of IL-1 $\beta$ , IL-6, and IL-23 in intestinal DCs and macrophages[17]. MiR-146a promotes M2 macrophage polarization by inhibiting Notch1 in RAW264.7 cells[18] and INHBA in monocytes from patients with systemic juvenile idiopathic arthritis[19].
miR-155	Elevated	TAB2 Socs1 Ship1 IL13RA1	MiR-155 modulates TLR/IL-1 signaling pathway in activated human monocyte-derived DCs by targeting TAB2[20]. The overexpression of miR-155 targeting Socs1 and Ship1 promotes pro-inflammatory cytokine production in LPS-stimulated DCs[21] and macrophages[22] in mice. MiR-155 targets IL13RA1 in human monocyte-derived macrophage and modulates IL-13 signaling cascade responsible for macrophage differentiation towards M2 phenotype[23].
miR-223	Elevated	NLRP3	MiR-223 limits NLRP3 inflammasome activation and suppresses the production of IL-1 $\beta$ and IL-18 both in mouse and human macrophages[24, 25]. MiR-223 modulates NF $\kappa$ B and MAPK signaling by targeting RhoB and suppresses pro-inflammatory cytokine production in LPS-stimulated macrophages[26].

DC, dendritic cell; INHBA, inhibin A subunit of activin A; IRAK1, interleukin-1 receptor-associated kinase 1; IL13RA1, interleukin-13 receptor subunit alpha-1; Irf4, interferon regulatory factor 4; NLRP3, NLR family pyrin domain containing 3; PTEN, phosphatase and tensin homolog; PDCD4, programmed cell death 4; RhoB, ras homolog gene family member B; RIPK2, receptor interacting serine/threonine kinase 2; Ship1, src homology 2 domain-containing inositol-5-phosphatase 1; Socs1, suppressor of cytokine signaling 1; TRAF6, TNF receptor associated factor 6; Tlr4, toll-like receptor 4; TAB2, transforming growth factor  $\beta$ -activated protein kinase 1-binding protein 2

**Supplementary Table S3.** Role of UC-associated miRNAs in adaptive immunity.

miRNA	Expression in UC	Target	Function
miR-21	Elevated	Il12a Il10	MiR-21 promotes Th2 differentiation by modulating IL-12 production from dendritic cells in mice[27]. MiR-21 acts as a potent negative regulator of IL-10-producing Breg differentiation in mice[28].
miR-29a/b	Elevated	IL12B Tbx21 Eomes	The NOD2-mediated upregulation of miR-29 suppresses IL-23 production in human dendritic cells by targeting IL-12p40 directly and IL-23p19 indirectly, and thereby inhibits Th17 differentiation[29]. IL-29 represses Th1 differentiation in mice by targeting Tbx21 (T-bet) and Eomes, inhibiting the production of IFN- $\gamma$ [30].
miR-31	Elevated	HIF1AN MAP3K14 SH2D1A Gprc5a	MiR-31 promotes Th1 differentiation by targeting HIF1AN, MAP3K14, and SH2D1A in primary human T cells[31]. TCR signaling-induced miR-31 negatively regulates peripherally-induced Tregs by targeting Gprc5a in mice[32].
miR-126	Elevated	Irs1	MiR-126 deficiency enhances the activation and proliferation capacity in T cells and promotes Th1/Th2 balance towards Th1 phenotype by upregulating IRS-1 expression in mice[33, 34].
miR-142	Elevated	Pde3b Socs1	MiR-142-5p plays a critical role in maintaining Treg suppressive function by repressing Pde3b expression in mice[35]. MiR-142a-5p overexpression shifted T cell differentiation towards Th1 phenotype[36].
miR-146a/b	Elevated	Stat1 Ncoa4	MiR-146a prevents Th17 differentiation in a rodent model of multiple sclerosis by reducing the production of IL-6 and IL-21 from auto-reactive T cells[37]. The downregulation of Stat1 by miR-146a is critical for the suppressor function of Tregs in mice[38]. MiR-146b may inhibit Th17 differentiation by targeting Ncoa4 and suppressing NCOA4-mediated PPAR $\gamma$ activation in mice[39]. MiR-146a may promote IL-10-producing Breg differentiation[40].
miR-155	Elevated	Jarid2 c-Maf Socs1	MiR-155 drives T cell differentiation towards Th17 lineage[21, 41]. MiR-155 promotes Th9/Th17 differentiation potentially by targeting c-Maf in mice[21]. MiR-155 promotes Treg proliferation by preventing SOCS1-dependent suppression of IL-2 receptor signaling in mice[42].

Eomes, eomesodermin; Gprc5a, G protein-coupled receptor class C group 5 member A; HIF1AN, hypoxia inducible factor 1 subunit alpha inhibitor; IRS1, insulin receptor substrate 1; Il10, interleukin-10; Il12a, interleukin-12a; IL12B, interleukin-12B; Jarid2, jumonji, AT-rich interaction domain containing 2; MAP3K14, mitogen-activated protein kinase kinase kinase 14; NCOA4, nuclear receptor coactivator 4; PPAR $\gamma$ , peroxisome proliferator-activated receptor Pde3b, phosphodiesterase 3 B; Breg, regulatory B cell; Treg, regulatory T cell; SH2D1A, SH2 domain containing 1A; Stat1, signal transducer and activator transcription 1; Socs1, suppressor of cytokine signaling 1; Tbx21, T-box transcription factor 21

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