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Original Article

Association of miR-10a expression and its rs3809783 polymorphism with postoperative incisional pain via Bcl-6/Tfh targeting in patients undergoing laparoscopic cholecystectomy

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Abstract: This study aimed to investigate the role of miR-10a and its rs3809783 polymorphism in postoperative incisional pain among patients undergoing laparoscopic cholecystectomy for acute cholecystitis (ACC-LC). Levels of miR-10a and immune markers (CD68, CD19, CD3, T-bet, GATA-3, IL-17A, Bcl-6) were measured in gallstone mucosal tissue, and genotyping was performed via PCR and sequencing. An incisional pain mouse model was used for mechanistic exploration. The results showed that miR-10a expression was decreased in ACC-LC patients compared with controls and was negatively correlated with pain intensity. Patients carrying the rs3809783 AT genotype exhibited lower miR-10a levels and higher pain scores than AA carriers. Among the elevated immune markers, only Bcl-6/Tfh was associated with pain severity. Further analysis confirmed Bcl-6 as a direct target of miR-10a, with the A→T substitution at rs3809783 enhancing Bcl-6-mediated Tfh polarization. This genotype was also correlated with longer anal exhaust time, higher inflammatory markers, and more complications. In conclusion, miR-10a and its rs3809783 polymorphism may serve as biomarkers for postoperative pain in ACC-LC by regulating the Bcl-6/Tfh pathway.

Keywords: MicroRNA, laparoscopic cholecystectomy, postoperative incisional pain, T follicular helper cells, polymorphism

Introduction

Laparoscopic cholecystectomy (LC) is the preferred surgical intervention for gallstone disease due to its advantages, including minimal trauma, reduced postoperative pain, shorter hospital stays, and less scarring [1]. Postoperative pain following LC primarily results from the inflammatory response triggered by surgical injury [2]. The immune system contributes to this process by releasing mediators that sensitize nociceptors and amplify pain perception [3]. In our previous work, we identified a series of immune cell-related genes associated with postoperative pain and inflammation in ACC-LC patients, notably finding that CXCR5 expression and its rs3922 polymorphism were closely linked to these outcomes. Given that CXCR5 is a marker of follicular helper T (Tfh) cells, these results suggest a potential involvement of Tfh cells in ACC-LC-associated pain [4].

Helper T cells comprise distinct subtypes: Th1 cells produce interferon-gamma (IFN-γ), which promotes cellular immune responses and contributing to nerve damage and pain, whereas Th2 cells exert anti-inflammatory effects that can alleviate pain [5, 6]. $CD4^+$ T cell reprogramming has been shown to effectively alleviate postoperative inflammatory pain, highlighting its strong potential for clinical translation [7].

Tfh cells, characterized by a $CXCR5^+PD\text{-}1^+ICOS^+Bcl\text{-}6^+$ phenotype and IL-21 secretion, regulate B cell responses in germinal centers and participate in inflammatory processes [8]. Dysregulated Tfh populations have been implicated in inflammatory diseases such as sys-

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temic lupus erythematosus and inflammatory bowel disease, where they drive pathogenic autoantibody production and local inflammation [8, 9]. Bcl-6 is a master transcription factor that orchestrates Tfh cell differentiation by directly activating Tfh signature genes (CXCR5, ICOS, PDCD1) while simultaneously repressing the lineage-specifying transcription factors (T-bet, GATA3, RORγt) and upstream receptors (IL-4Rα, IL-12Rβ2) of competing Th1, Th2, and Th17 differentiation programs. This dual regulatory function establishes BCL6 as the central determinant of Tfh cell fate [10].

MicroRNAs also modulate T cell polarization. Among them, the immunoregulatory functions of miR-10a have been well documented in inflammatory diseases, where its dysregulation correlates with altered Treg frequencies and cytokine profiles [11], and it inhibits dendritic cell activation and suppresses Th1/Th17 responses [12]. Recently, the single nucleotide polymorphism (SNP) rs3809783 (A→T) has been reported to affect miR-10a expression, thereby influencing disease susceptibility [13, 14].

In this study, we examined miR-10a expression, its rs3809783 polymorphism, and Tfh-related markers in ACC-LC patients. We observed reduced miR-10a expression and elevated Bcl-6 levels in gallstone-associated mucosal tissues. Importantly, the rs3809783 AT genotype correlated with lower miR-10a expression, higher Bcl-6 levels, and greater postoperative incisional pain. Mechanistic assays further demonstrated that Bcl-6 is a direct target of miR-10a and that miR-10a modulates pain through inhibition of the Bcl-6/Tfh axis. Collectively, these findings reveal a novel regulatory pathway in postoperative pain and support the potential for personalized pain management strategies based on T cell phenotypes.

Materials and methods

Subjects

This study builds upon our previously published research, and the patient cohort as well as the enrollment and exclusion criteria were consistent with that earlier work [4]. In brief, a total of 60 individuals diagnosed with ACC-LC and treated at our hospital were recruited. The eligibility requirements included: (1) Confirmation of gallstone disease with consent to undergo surgery, and (2) Adequate literacy and normal communication ability. Patients were excluded if they had (1) Malignant tumors, (2) Cognitive impairment, (3) A history of prior gallbladder surgery, (4) Combined hepatic or biliary disorders, or (5) Psychiatric illness. The negative control (NC) group consisted of 15 samples from patients undergoing cholecystectomy due to gallbladder polyps or adenomyosis. These patients had localized disease distant from the gallbladder and no history of biliary obstruction.

Specific marker levels

The levels of specific protein markers in tissue samples were measured by immunohistochemical staining, following the protocols and quantitative methods described in previous publications [4, 13].

Quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

Total RNAs were reverse transcribed into cDNAs using a cDNA Synthesis Kit (TaKaRa, Osaka, Japan), and the cDNAs were amplified using the SYBR Premix Ex Taq II Kit (TaKaRa, Osaka, Japan). miR-10a levels were measured using the LightCycle®96 Real-time PCR System (Roche, Basel, Switzerland).

Genotypes

rs3809783 genotyping was performed by sequencing, following the method described by Li et al., and the method description partly reproduces their wording [14]. Genomic DNA was extracted from patients' whole blood using the TIANamp Blood DNA Kit (TIANGEN, Beijing, China). The pri-miR-10a region of each DNA sample was amplified by PCR using the following primers: 5'-CCCTTCTCCTATCCACTCCT-3' and 5'-AAAGCACTCAAACCACACCC-3'. The resulting PCR fragments were then sequenced in the forward direction.

Cell culture

HEK 293T cells were cultured in DMEM medium supplemented with 10% FBS at 37°C in a 5% CO_2 atmosphere. For primary $CD4^+$ T cells, we isolated them from peripheral blood mono-

nuclear cells (PBMCs) obtained from a healthy volunteer, using the Dynabead Separation Kit, according to a previous report [15].

Cell transfection

miR-10a mimic, miR-10a inhibitor, agomiR-10a, and their corresponding negative controls were all purchased from Ruibo (Guangzhou, China). Cells were transfected using the Neon electroporation transfection system (Invitrogen).

Western blotting assay

Proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis, transferred onto immobilon PVDF membranes (Bio-Rad), and probed with anti-Bcl-6 antibody (Abcam, UK, ab190190) and anti-β-actin antibody (Abcam, UK, ab8227), followed by chemiluminescent detection. Band intensities were quantified using ImageJ software (NIH, Bethesda, MD, USA), and the relative protein expression levels were normalized to the corresponding β-actin signal. Full scan of western blotting gel used in this study was shown in Supplementary Figure 1.

Flow cytometry

Cells were fixed and stained for intracellular markers, including anti-CD3, anti-CD4, anti-PD-1 and anti-CXCR5. Samples were analyzed by BD LSR II flow cytometer (Becton Dickinson). The gating strategy was as follows: lymphocytes were first identified by forward scatter (FSC) and side scatter (SSC), followed by selection of $CD3^+CD4^+$ cells, and then $CD4^+$ T cells were further analyzed for PD-1 and CXCR5 expression to identify Tfh cells ($CD3^+CD4^+CXCR5^+PD\text{-}1^+$). Samples were analyzed by BD LSR II flow cytometer (Becton Dickinson). At least 10,000 events were acquired for each sample.

Mice and incisional pain model

Wild-type C57BL/6J mice, 7-8 weeks old and maintained under specific pathogen-free (SPF) standard, were used for this study. The incisional pain model was established by making a 5-mm longitudinal incision on the plantar surface of the right hind paw using a No. 11 surgical blade, according to a previous report [16]. Mechanical and thermal hypersensitivity behaviors were monitored in each mouse to reflect the level of pain. To impair Tfh cell function, mice were injected intraperitoneally with 25 μg of the BCL6 BTB domain inhibitor FX1 (Merck) or with an equal volume of vehicle as a control every two days, together with agomiR-10a at 10 mg/kg daily (starting 1 day after surgery) until the end of the experiment. For the animal experiments, mice were randomly assigned to treatment groups using a computer-generated randomization schedule. All behavioral tests were performed by an investigator blinded to the group assignments, and data analysis was also conducted in a blinded manner.

Statistical analysis

Results are expressed as the mean ± standard deviation. Normality of all data was assessed prior to statistical analysis. Differences between the two groups were analyzed using Student's t-test in SPSS Statistics for Windows, v.24 (IBM Corp., Armonk, NY, USA) when the data were normally distributed. For non-normally distributed continuous variables, the Mann-Whitney U-test was applied. Pearson's chi-squared test was used to compare discrete variables between the two groups. Statistical significance was set at $P < 0.05$. All in vitro experiments were performed with at least three biological replicates, and animal experiments included at least six mice per group.

Results

Association between miR-10a, monocytes/macrophages, B cells, T cells and specific helper T cell subtypes and the incision pain levels in ACC-LC patients

Using the same patient cohort as in our previous study, we observed that miR-10a expression in gallstone-associated mucosal tissues was significantly lower than that in NC tissues (**Figure 1A**). Concurrently, the expression of CD68 (a monocyte/macrophage marker), CD19 (a B cell marker), and CD3 (a T cell marker) was markedly higher in gallstone mucosa compared with NC tissues (**Figure 1B**). T cell subsets include helper T cells (Th1, Th2, Th17 and Tfh), regulatory T cells (Tregs), cytotoxic T cells, memory T cells, and gamma-delta T cells (γδ T cells) [17], with helper T cells reported to play a pivotal role in pain modulation [18]. Here,

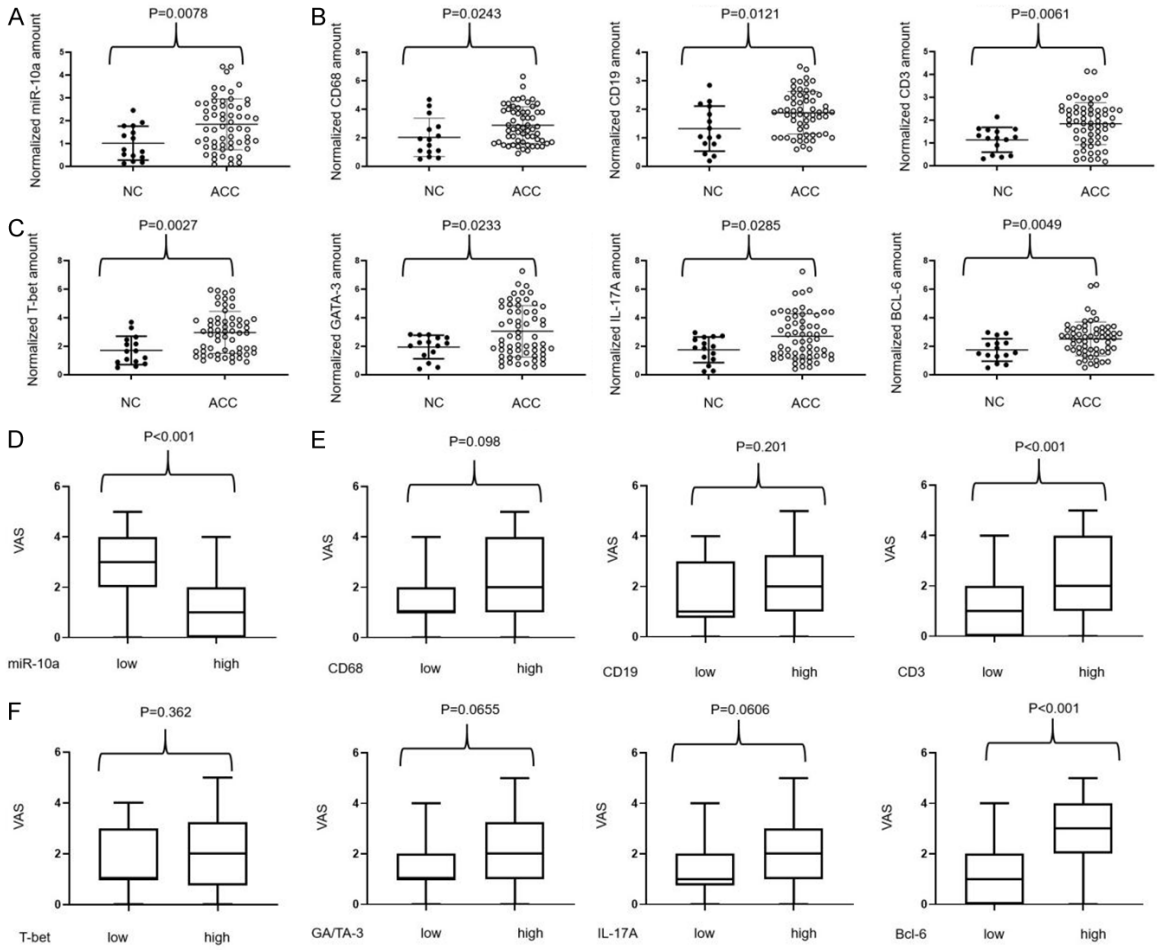

Figure 1. Association between miR-10a, monocytes/macrophages, B cells, T cells, and specific helper T cell subtypes and incision pain levels in ACC-LC patients. A. The expression levels of miR-10a were determined by qRT-PCR, and were compared between mucosal tissue of gallstones and the NC tissues. B. The expression levels of CD68; CD19 and CD3 were determined by immunohistochemistry stain, and were compared between mucosal tissue of gallstones and the NC tissues. C. The expression levels of T-bet; GATA-3; IL-17A and Bcl-6 were determined by immunohistochemistry stain, and were compared between mucosal tissue of gallstones and the NC tissues. D. VAS were compared between the ACC-LC patients with the miR-10a low and high phenotype. E. VAS were compared between the ACC-LC patients with CD68 low and high phenotype; CD19 low and high phenotype and CD3 low and high phenotype. F. VAS were compared between the ACC-LC patients with T-bet low and high phenotype; GATA-3 low and high phenotype; IL-17A low and high phenotype and Bcl-6 low and high phenotype.

we assessed the expression of T-bet (Th1 marker), GATA-3 (Th2 marker), IL-17A (Th17 marker), and Bcl-6 (Tfh marker), and found that all were upregulated in gallstone mucosal tissues (**Figure 1C**).

We stratified the 60 ACC-LC patients into high- and low-miR-10a expression groups using the median expression as the cutoff. Patients with high miR-10a expression exhibited lower VAS scores compared to the low-expression group (**Figure 1D**). Additionally, patients with high CD3 or Bcl-6 expression had higher VAS scores than those with low expression, whereas correlations between VAS scores and the other markers did not reach statistical significance (**Figure 1E**, **1F**). Collectively, these results further corroborate the close association between Tfh cells and ACC-LC-related postoperative pain.

Association between miR-10a and rs3809783 polymorphism and Bcl-6/Tfh in patients with ACC-LC

Considering that miR-10a was negatively correlated with VAS scores, whereas CD3 and Bcl-6 were positively correlated with VAS scores, we

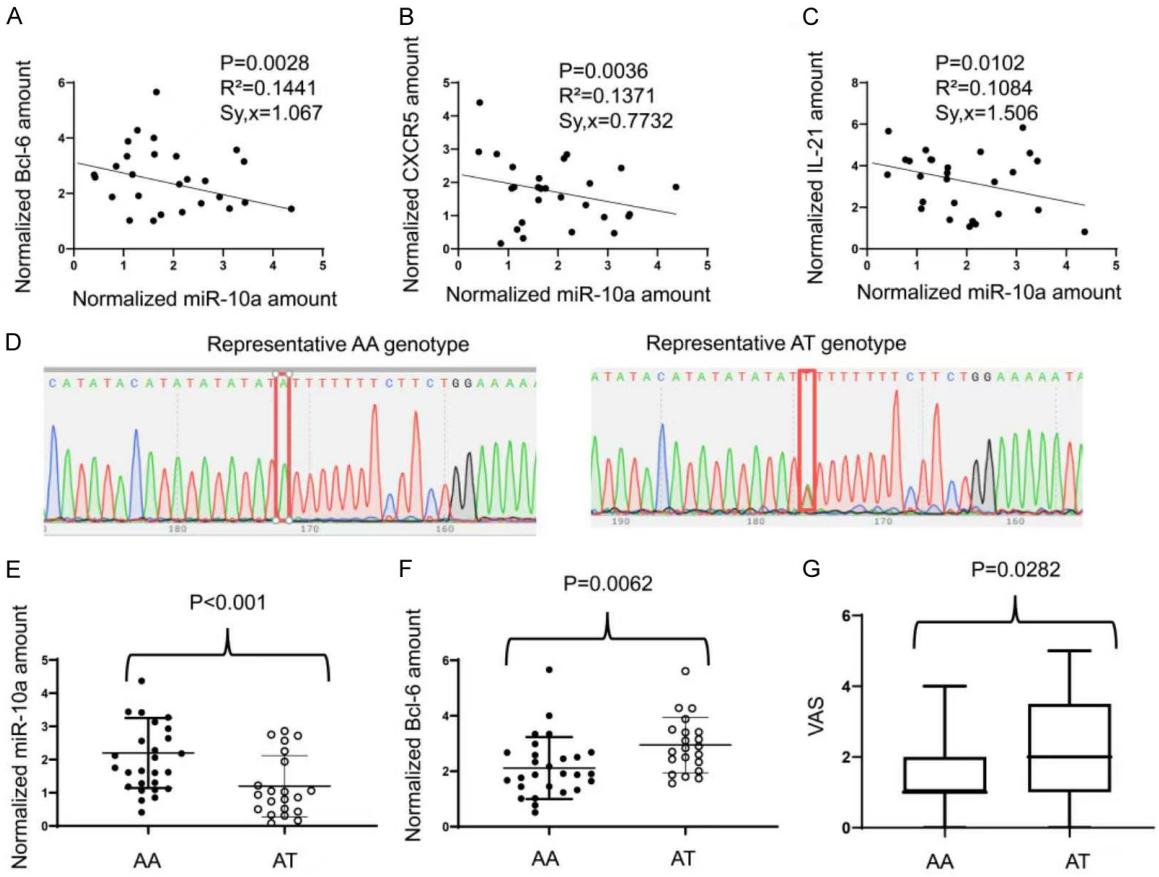

Figure 2. Association between miR-10a, rs3809783 polymorphism and Bcl-6/Tfh in patients with ACC-LC. (A-C) Correlation between miR-10a levels and (A) Bcl-6 levels; (B) CXCR5 levels; and (C) IL-21 levels in the mucosal tissue of gallstones in patients with ACC-LC. (D) Representative sequencing chromatograms for the rs3809783 AA and AT genotypes from the patients in this study. (E-G) The expression levels of (E) miR-10a and (F) Bcl-6, as well as (G) VAS were compared between rs3809783 AA genotype group and AT/TT genotype group.

next examined the relationship between miR-10a and T cell markers. We found that miR-10a expression was negatively correlated with Bcl-6 (**Figure 2A**) but showed no correlation with T-bet, GATA-3, or IL-17A (data not shown). Additional key Tfh markers, including CXCR5 and IL-21, were also negatively associated with miR-10a levels (**Figure 2B**, **2C**). These findings indicate a close relationship between miR-10a and the Bcl-6/Tfh axis in ACC-LC patients.

It is well established that miR-10a expression is influenced by genetic factors, particularly the rs3809783 A-to-T polymorphism, which has been reported to impair the maturation of miR-10a [14]. Among the 60 ACC-LC patients, 39 carried the AA genotype, whereas 21 carried the AT genotype. No patients with the TT genotype were identified in the clinical samples of this study. Representative sequencing chromatograms for the AA and AT genotypes are shown in **Figure 2D**. Patients with the AT genotype exhibited lower miR-10a levels (**Figure 2E**), higher Bcl-6 expression (**Figure 2F**), and higher VAS scores (**Figure 2G**). These results suggest that the miR-10a rs3809783 A/T polymorphism may serve as a predictor of postoperative incisional pain in ACC-LC patients through its regulation of the miR-10a/Bcl-6/Tfh axis.

Bcl-6 is a promising target of miR-10a in T cells

Using the TargetScan program [19], we identified Bcl-6 as a potential miR-10a target (**Figure 3A**). To provide direct evidence for this, we generated two luciferase reporter vectors that contained the putative miR-10a binding sites within the 3'UTR and mutant 3'UTR (3'MUT). These two luciferase reporters were transfect-

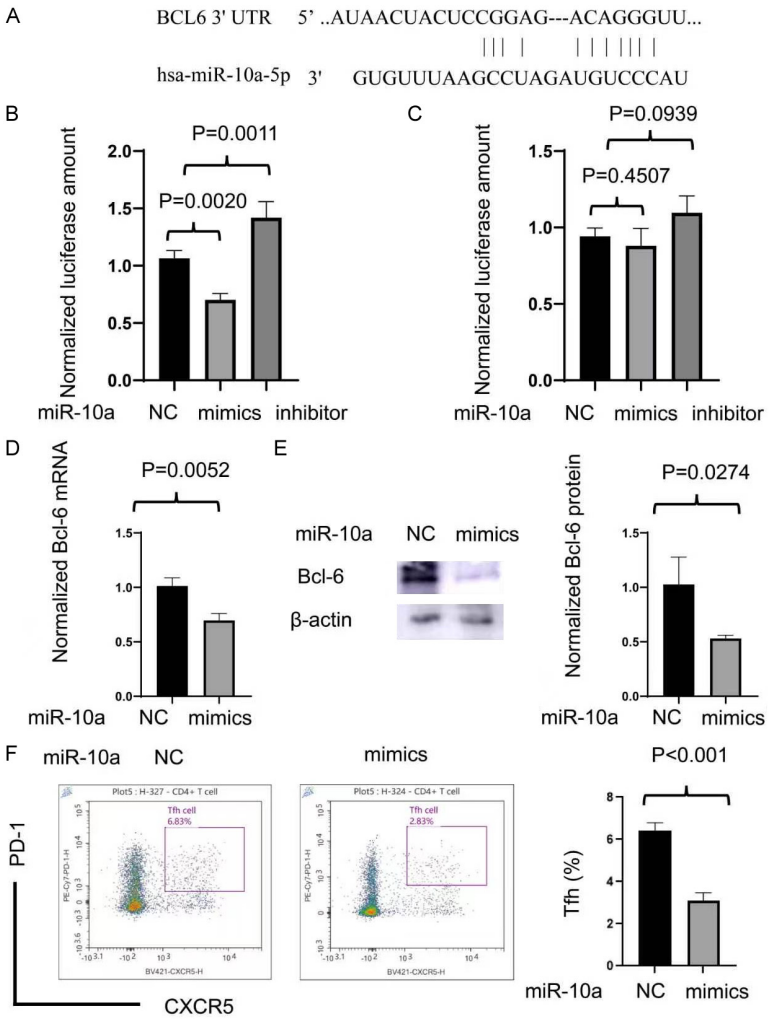

Figure 3. Bcl-6 is a promising target of miR-10a in T cells. (A) The region of the human Bcl-6 mRNA 3'UTR predicted to be targeted by miR-10a, as generated by the TargetScan program. (B, C) HEK-293T cells were transiently cotransfected with (B) Bcl-6 3'UTR luciferase report vectors, or (C) mutant Bcl-6 3'UTR luciferase reporter vectors (3'MUT), and either miR-10a mimics, miR-10a inhibitor or miR-control (NC). Luciferase activities were normalized to Renilla luciferase activity. (D-F) Circulating $CD4^+$ cells from healthy donors were cultured and transfected with miR-10a mimics or miR-control (NC), and the (D) Bcl-6 mRNA levels were determined by RT-qPCR; (E) Bcl-6 protein levels were determined by western blotting assay. Left: Representative western blot images; Right: Quantitative results of western blotting assay. (F) Tfh proportions were determined by flow cytometry. Left: Representative flow cytometry plots; Right: Quantitative results of flow cytometry.

ed into human 293T cells together with an miR-10a mimic or inhibitor. For the 3'UTR reporters, miR-10a mimics inhibited, while the miR-10a inhibitor enhanced the luciferase activity (**Figure 3B**). For the 3'MUT reporters, little effect of miR-10a mimics or the miR-10a inhibitor was found on the luciferase activity (**Figure 3C**). Circulating $CD4^+$ T cells from healthy donors were purified by magnetic bead separation. We transfected the cultured $CD4^+$ T cells with miR-10a mimics or a control and found that Bcl-6 mRNA and protein levels were downregulated (**Figure 3D**, **3E**). In addition, the percentage of Tfh cells was significantly decreased in the miR-10a transfected group compared with the control group (**Figure 3F**).

A→T substitution in miR-10a rs3809783 inhibits miR-10a levels and promotes Bcl-6/Tfh

Next, we constructed miR10a-AA, miR10a-TT, and miR10a-AT plasmids according to a previous report [14], and transfected them into the cultured $CD4^+$ T cells. miR-10a levels were lower in the miR10a-TT/AT group than in the miR10a-AA group (**Figure 4A**). Meanwhile, Bcl-6 mRNA and protein levels were both higher in the miR10a-TT/AT group (**Figure 4B**, **4C**). In addition, the percentage of Tfh cells was significantly increased in the miR10a-TT/AT group (**Figure 4D**). These results indicated that the A→T substitution at rs3809783 reduces miR-10a levels and promotes Bcl-6/Tfh.

Attenuating effect of miR-10a on incisional pain in mice

We next employed a mouse model of incisional pain to investigate the role of the miR-10a/Bcl-6 axis in pain regulation. Paw withdrawal threshold (PWT) and thermal withdrawal latency (TWL) responses were evaluated. At baseline, no significant differences in PWT or TWL values were observed between the NC group and the other groups. Following plantar incision, PWT and TWL values decreased significantly in all groups

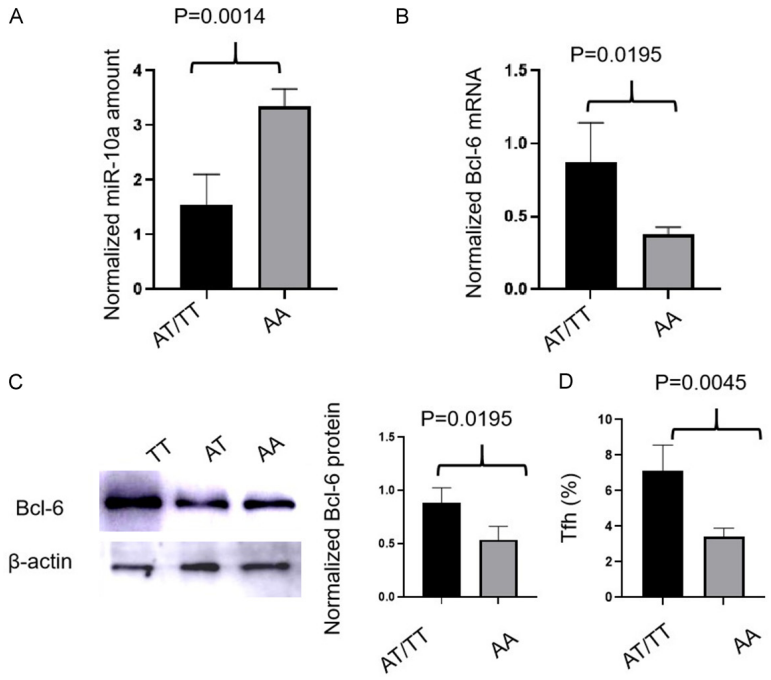

Figure 4. A to T substitution in miR-10a rs3809783 inhibits miR-10a levels and promotes Bcl-6/Tfh. (A-D) pCR3.1-miR-10a-TT, pCR3.1-miR-10a-A/T or pCR3.1-miR-10a-AA was transfected into circulating $CD4^+$ cells. After 48 h of transfection, (A) miR-10a levels were determined by RT-qPCR; (B) Bcl-6 mRNA levels were determined by RT-qPCR; (C) Bcl-6 protein levels were determined by western blotting. Left: Representative western blot images; Right: Quantitative results. (D) Tfh cell proportions were determined by flow cytometry. Quantitative results are shown.

and remained relatively low thereafter. At 24 hours post-modeling, treatment with FX1, agomiR-10a, or FX1 combined with agomiR-10a was initiated. The results showed that, compared with the untreated control group, all treatment groups exhibited significantly increased PWT and TWL values on days 4 and 7 post-modeling (**Figure 5A**, **5B**). In the presence of FX1 (i.e., after Tfh cells had been suppressed), no significant differences in PWT or TWL values were observed between the agomiR-10a group and the group without agomiR-10a (**Figure 5A**, **5B**). Collectively, these findings indicate that miR-10a attenuates incisional pain in mice by targeting Bcl-6 to regulate Tfh cells.

Association between rs3809783 polymorphism and perioperative clinical features in patients with ACC-LC

Next, we examined the correlation between the rs3809783 polymorphism and perioperative clinical features in patients with ACC-LC. Among the gastrointestinal function indicators, anal exhaust time was longer in the rs3809783 AT group than in the rs3809783 AA group. However, no significant differences were observed between the two groups in terms of recovery time of bowel sounds, time to first defecation, or time to first food intake (**Table 1**).

With regard to inflammatory indicators, both PCT and CRP levels at 24 h after surgery were significantly higher in the rs3809783 AT group than that in the rs3809783 AA group (**Table 1**). We also found that the total number of complications, including incisional infections, intestinal adhesions, bile leakage, and gastrointestinal reactions, were higher in the AT group than in the AA group (**Table 1**).

Discussion

Pain in patients with ACC-LCs is a common concern and can result from various factors. A primary cause of ACC-LC associated pain is excessive inflammation secondary to immune system dysfunction. T cells play a central role in the immune response [5, 6]. These cells infiltrate the gallbladder tissue, contributing to the inflammation that leads to tissue damage and pain.

Biomarkers can help identify disease subtypes and define diagnostic categories and treatments based on pathophysiology. However, the diagnosis and treatment of pain still rely primarily on self-reported symptoms, as biomarkers for pathophysiological mechanisms that support individualized treatment are currently unavailable. Notably, pain biomarkers are not intended to replace self-reported pain but to identify biological processes associated with pain. Understanding these processes can help reveal the underlying causes of pain, improve diagnosis and treatment, and drive the development and application of novel therapeutics [20].

In this study, we identified miR-10a and its rs3809783 polymorphism as potential bio-

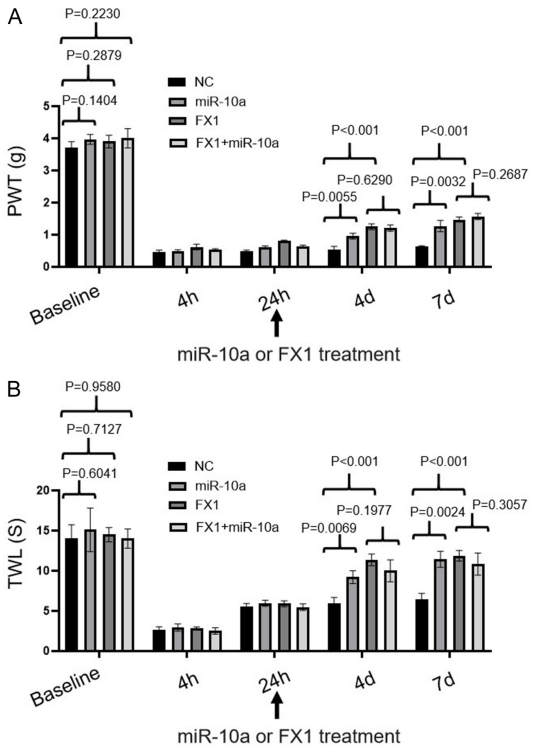

Figure 5. Attenuating effect of miR-10a on incisional pain in mice through Bcl-6/Tfh targeting. (A, B) Incisional pain wild-type mice were treated with FX1 at 25 μg/mouse or an equal volume of vehicle (NC) every two days, together with agomiR-10a at 10 mg/kg daily (starting 24 hours after modeling), followed by behavioral testing. (A) Paw withdrawal threshold (PWT) and (B) thermal withdrawal latency (TWL) were compared between the agomiR-10a treated group and the control group.

markers of ACC-LC associated pain. We found that the miR-10a rs3809783 A→T substitution decreased miR-10a levels and therefore enhanced the VAS values. We also found that the rs3809783 AT genotype correlated with adverse outcome indicators in patients with ACC-LC, including prolonged anal exhaust time, elevated inflammatory markers, and higher total complication rate factors, which are often linked to poorly managed postoperative pain [21]. These findings uncover genetic mechanisms that explain individual differences in pain and offer new approaches for predicting and managing pain in clinical practice.

Importantly, it must be emphasized that the clinical observations in this study are correlational in nature, and causality cannot be inferred from the patient data alone. Our findings in ACC-LC patients establish a clear association between miR-10a rs3809783 genotype, miR-10a expression, Bcl-6/Tfh levels, and postoperative pain severity; however, observational studies inherently cannot exclude confounding factors or reverse causation. To move beyond correlation and test the causal relationship, we performed complementary in vitro functional assays and in vivo intervention experiments. Mechanistically, we found that this association may be due to regulation of Bcl-6 by miR-10a during the process of pain. Bcl-6 is a transcription factor known to promote Tfh cell development by inhibiting genes linked to alternative T cell fates, such as Th1, Th2, and Th17, thereby ensuring Tfh cell formation [22]. During inflammatory immune responses, Tfh cells are crucial for supporting B cells within germinal centers, facilitating the production of high-affinity antibodies. They provide essential signals, such as IL-21 and CD40L, to B cells, promoting proliferation, somatic hypermutation, and class-switch recombination, all of which are vital for effective antibody responses [23]. In addition, Tfh cells have been shown to promote T cell-mediated inflammation [7]. Therefore, we speculated that Bcl-6/Tfh is likely closely related to inflammatory pain, although there are currently no reports in this regard. Here, we found that miR-10a targeted Bcl-6 directly in T cells, and that miR-10a decreased incisional pain in wild-type mice; however, this effect was not observed under Bcl-6 inhibition, confirming that miR-10a could reduce incisional pain through Bcl-6 inhibition.

Bcl-6/Tfh cells also play a role in the regulation of immune responses to prevent excessive inflammation. For example, Yu et al. demonstrated that Bcl-6 negatively regulates macrophage proliferation by suppressing the autocrine production of IL-6 [24]. Moreover, Bcl-6 helps modulate the immune response by inhibiting the differentiation of T cells into highly inflammatory subsets; this balances the need for a strong immune response with the necessity of limiting inflammation-induced tissue damage [25]. This evidence suggests that Bcl-6/Tfh cells may have a bidirectional role in regulating inflammation at various stages of different diseases. Therefore, although miR-10a can inhibit Bcl-6 expression, the specific molecular mechanism of the miR-10a/Bcl-6 axis in incisional pain may be complex and warrants further investigation.

Table 1. The correlation between different clinical parameters and Bcl-6 amount in ACC-LC patients

Parameter	rs3809783 polymorphism		
	AA (n=39)	AT (n=21)	
Gastrointestinal function indicator			
Recovery time of bowel sounds (hours)	14.13±2.11	15.24±2.15	0.0620
Anal exhaust time (hours)	22.27±2.34	23.74±2.36	0.0261
Anal defecation time (hours)	36.80±4.85	38.19±5.98	0.3664
First eating time (days)	2.41±0.45	2.50±0.36	0.4026
Inflammatory indicator			
24 hours postoperative serum PCT (μg/L)	1.40±0.31	1.59±0.23	0.0095
24 hours postoperative serum CRP (mg/L)	10.08±2.97	11.75±2.53	0.0264
Complication indicator			
Incision infection (Y/N)	2/37	4/18	
Intestinal adhesions	0/39	1/20	
Gastrointestinal reactions	2/36	3/18	
Total of complication (n)	4	8	0.0410

Several limitations of this study should be acknowledged. First, the relatively small sample size and the single-center design may limit the generalizability and statistical power of our findings, and we acknowledge that these factors weaken the robustness of our conclusions to some extent. Therefore, we emphasize that further validation in larger cohorts from multiple centers is warranted to confirm our results.

Second, this study used a general plantar incisional pain model in mice, as no ACC-LC specific animal model currently exists. It is important to recognize that this model primarily recapitulates the acute postoperative inflammatory response and peripheral nociceptor sensitization that follow surgical tissue injury-mechanisms that are also central to incisional pain in ACC-LC patients. However, it does not reproduce the chronic gallbladder inflammation, local immune cell infiltration, or the specific biliary pathology that characterize ACC-LC. Consequently, while this model is appropriate for validating the conserved molecular pathway (miR-10a → Bcl-6 → pain behavior) in a controlled setting, it cannot fully capture the disease-specific context in which this pathway is altered. Despite this limitation, the convergence of our clinical and animal data-particularly the consistent negative correlation between miR-10a and pain severity across both settings, and the loss of the agomiR-10a effect under Bcl-6 inhibition-strengthens the argument that the miR-10a/Bcl-6/Tfh axis is functionally relevant to postoperative pain in humans. Future development of ACC-LC-relevant animal models, such as those incorporating pre-existing gallbladder inflammation or biliary tract sensitization, would be valuable to further validate the translational significance of our findings.

Third, it should also be acknowledged that the mechanistic link between Tfh cells and postoperative pain remains largely correlative in nature; although our clinical, in vitro, and in vivo data collectively support a functional role for the miR-10a/Bcl-6/Tfh axis in pain modulation, direct evidence linking Tfh cells themselves to nociceptor sensitization is still indirect, and we cannot exclude the contribution of other Bcl-6-expressing cells or Tfh-related cytokines (e.g., IL-21). Future studies employing Tfh-cell-specific depletion or adoptive transfer models, along with longitudinal perioperative analyses, are warranted to establish definitive causality and to determine whether Tfh cells or their secreted products are the primary drivers of postoperative inflammatory pain.

Conclusion

miR-10a and its rs3809783 polymorphism may serve as potential biomarkers for postoperative incisional pain in patients with ACC-LC.

Disclosure of conflict of interest

None.

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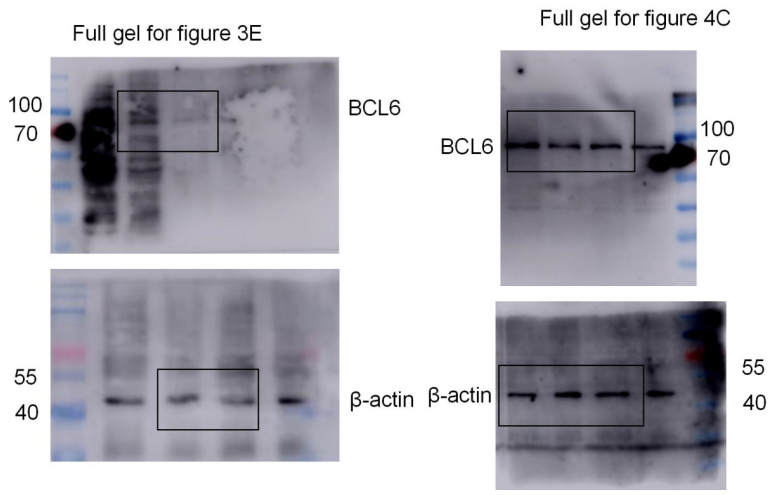

Supplementary Figure 1. Full scan of western blotting gel used in this study.