Original Article
miR-551b-5p increases intracellular Ca^{2+} concentration but does not alter c-Kit expression in rat interstitial cells of Cajal

Hua-Jing Ke[1*], Ji Li[1*], Xiao-Juan Yang[1], Wang Zhang[1], Tao Su[2], Tan-Wei Xiong[3], Jun Song[1], Pi Liu[1]

[1]Department of Gastroenterology, The First Affiliated Hospital of Nanchang University, Nanchang, China; [2]Department of Gastroenterology, The Third Hospital of Jingdezhen, Jingdezhen, China; [3]Department of Gastroenterology, Guilin Medical University, Guilin, China. *Equal contributors.

Abstract: Objective: To investigate the effect of the severe acute pancreatitis (SAP)-related miR-551b-5p on intracellular Ca^{2+} concentration and c-Kit expression and distribution in rat interstitial cells of Cajal (ICCs) *in vitro*. Methods: ICCs were isolated from 5-10-day-old rats and cultured *in vitro*. The cultured ICCs were divided into five groups: a normal control group; a group transfected with an miR-551b-5p mimic; a group transfected with an miR-551b-5p inhibitor; a group transfected with a negative control for the miR-551b-5p mimic; and a group transfected with a negative control for the miR-551b-5p inhibitor. After transfection, real-time PCR was used to detect miR-551b-5p and c-Kit expression. A Western blot analysis was used to determine the expression of c-Kit protein. Confocal microscopy combined with immunofluorescence and Fluo 3-acetoxymethyl (AM) fluorescence were used to determine the localization of c-Kit and intracellular Ca^{2+} concentration, respectively. Results: Transfection with the miR-551b-5p mimic or inhibitor resulted in overexpression or downregulation of miR-551b-5p in ICCs, respectively. The overexpression or downregulation of miR-551b-5p had no significant influence on c-Kit mRNA or protein levels. The overexpression of miR-551b-5p significantly increased the intracellular Ca^{2+} concentration, and the downregulation of miR-551b-5p significantly decreased the intracellular Ca^{2+} concentration. Conclusion: miR-551b-5p increases intracellular Ca^{2+} concentration but does not alter c-Kit expression in rat ICCs, suggesting that it functions in ICCs by regulating the intracellular Ca^{2+} concentration downstream or independently of c-Kit signaling.

Keywords: microRNA, c-Kit, ICCs, acute pancreatitis, gastrointestinal motility disorder

Introduction

Severe acute pancreatitis (SAP) is a common, life-threatening disease characterized by acute onset, rapid progression, multiple complications, and high mortality [1, 2]. SAP often leads to the development of gastrointestinal motility disorder (GID) in the early stage of the disease [3], which can cause paralytic ileus, abdominal compartment syndrome, gastrointestinal barrier dysfunction, and bacterial and endotoxin translocation. This can result in infectious complications, systemic inflammatory response syndrome, and multiple organ failure [4-6]. Therefore, GID is an important phase in the pathogenesis, progression, and prognosis of SAP [7-9]. Elucidating the mechanisms underlying GID is of great clinical importance to understand the pathogenesis of SAP and to develop novel treatments for this disease.

Gastrointestinal motility is closely associated with the physiological functions of interstitial cells of Cajal (ICCs), a special class of interstitial cells that are found in the gastrointestinal tract. ICCs serve as important pacemakers in the establishment of slow waves and control of smooth muscle contraction [10]. Loss or injury of ICCs has been found to contribute to motor pathologies observed in a number of gastrointestinal diseases in humans, such as slow-transit constipation, pseudo-obstruction, and diabetic enteropathy [10, 11]. Interestingly, changes in the numbers and ultrastructure of ICCs have also been observed in experimental SAP in rats [12-14], suggesting that altered

physiological functions of ICCs may be involved in the pathogenesis of SAP-associated GID.

Several mechanisms have been found to regulate the pacemaker activity of ICCs in the gastrointestinal tract. Huizinga *et al* [11] reported that c-Kit, a receptor tyrosine kinase, was required for the ICC pacemaker activity. Blockage of the c-Kit signaling pathway resulted in decreased numbers of c-Kit-positive cells and ultrastructural changes in ICCs in rats with SAP [12]. In addition, the intracellular Ca^{2+} concentration, which is controlled by the endoplasmic reticulum and mitochondria, is essential for the instantaneous pacemaker current of ICCs [15, 16]. Given the frequent presence of GID and potentially disrupted physiological functions of ICCs in SAP, changes in the c-Kit signaling pathway and intracellular Ca^{2+} concentration may be involved in the pathogenesis of SAP-associated GID.

MicroRNAs (miRNAs) are a class of small non-coding RNA molecules that regulate gene expression at the posttranscriptional level. The dysregulation of miRNAs is closely associated with many human diseases [17]. Intriguingly, miRNAs such as miRNA-510, miRNA-221, and miRNA-222 have been found to be related to SAP-associated GID, c-Kit, and intracellular Ca^{2+} concentration regulation [15, 16]. In a previous study, we found that miR-551b-5p was differentially expressed in rats with SAP compared to those with mild acute pancreatitis, and that it could be used to predict the severity of SAP [17]. However, its mechanism of action in SAP is still unknown. In this study, we overexpressed and downregulated miR-551b-5p in ICCs to assess the influence of these changes on c-Kit expression and intracellular Ca^{2+} concentration. Our findings offer new clues regarding the pathogenesis of SAP-associated GID.

Materials and methods

Preparation and culture of ICCs

ICCs were prepared and identified following a previously described protocol [18]. Briefly, Sprague-Dawley rats (5-10 days old) were killed and fully soaked in 75% ethanol for 5 min. The abdominal cavities of the rats were then opened under sterile conditions, and small intestinal tissue was harvested and placed in phosphate-buffered saline (PBS; Boster, Wuhan, China) containing 100 U/mL penicillin

and 100 µg/mL streptomycin. Under a dissecting microscope, the small intestine mesentery, blood vessels, and serosal layer were removed, followed by sharp dissection of the layers of the mucosa and submucosa. The remaining smooth muscle layer was cut into pieces and digested using 0.2% collagenase II solution (Boster) for 30 min at 37°C. Cells were dissociated by repeated pipetting and filtered using a 100-gauge stainless steel mesh. The cell pellet was re-suspended in Dulbecco's modified Eagle's medium (DMEM; Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum (Gibco) and 1% penicillin-streptomycin solution (Boster), and then cultured at 37°C in an atmosphere containing 5% CO_2.

ICC groups and transfection

The ICCs cultured *in vitro* were divided into five groups: a normal control group; an miR-551b-5p overexpression group (transfected with an miR-551b-5p mimic); a negative control group for the miR-551b-5p mimic group; an miR-551b-5p downregulation group (transfected with an miR-551b-5p inhibitor); and a negative control group for the miR-551b-5p inhibitor group. Cell transfection was performed using Lipofectamine 2000 Transfection Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The miR-551b-5p mimic and inhibitor were obtained from Novartis (Shanghai, China).

Real-time PCR

Total RNA was prepared from the ICCs using TRIzol Reagent (Invitrogen). Reverse transcription was carried out using Moloney Murine Leukemia Virus (M-MLV) reverse transcriptase (Progema, Madison, WI, USA). Subsequently, real-time PCR was performed in a reaction system containing SYBR Green/Fluorescein qPCR Master Mix (2×) (Fermentas, Waltham, MA, USA) and Ex TaqTM (TaKaRa, Japan) with an EDC-810 PCR thermal cycler (Eastwin, Beijing, China) as described previously [19]. The PCR cycling parameters were as follows: one cycle of 50°C for 2 min and 95°C for 10 min, followed by 40 cycles of 95°C for 30 s and 60°C for 30 s. The expression levels of miR-551b-5p and c-Kit were determined relative to U6 and β-actin, respectively. The primers were synthesized by Genescript (Nanjing, China) as follows: for miR-551b-5p: forward, 5'-TGC GCG AAA TCA AGC TTG GGT G-3' and reverse, 5'-CTC

AAG TGT CGT GGA GTC GGC AA-3'; for rat c-Kit (276-bp product): forward, 5'-TTG GCA AAG AAG ACA ACG AC-3' and reverse, 5'-GCA CAG ACA CCA CTG GGA TA-3'; for β-actin (240-bp product): forward, 5'-CAC GAT GGA GGG GCC GGA CTC ATC-3' and reverse, 5'-TAA AGA CCT CTA TGC CAA CAC AGT-3'; and for U6: forward, 5'-CGC TTC GGC AGC ACA TAT AC-3' and reverse, 5'-AAA TAT GGA ACG CTT CAC GA-3'. Each assay was repeated in triplicate.

Western blot analysis

The expression of c-KIT protein in the ICCs was detected using a Western blot analysis. Briefly, the ICCs were lysed in radioimmunoprecipitation assay (RIPA) lysis buffer to yield whole-cell lysates. After determination of the protein concentrations, equal amounts of protein (50 μg) were resolved by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto polyvinylidenedifluoride (PVDF) membranes (Millipore, Bedford, MA, USA). After blocking with normal goat serum (Boster), the membranes were incubated first with primary mouse monoclonal anti-c-Kit antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA) or anti-β-actin antibody (Boster) and then with horseradish peroxidase (HRP)-conjugated goat anti-mouse secondary antibody (Boster). The membranes were then developed using an enhanced chemiluminescence (ECL) system (Thermo Scientific Pierce, USA), and the density of each band was measured using BandScan software (Glyko, Novato, CA, USA). Each assay was repeated in triplicate.

Immunofluorescence, intracellular Ca^{2+} measurement, and confocal microscopy

The localization of c-Kit was determined using immunofluorescence and confocal microscopy. Briefly, the adherent cells on coverslips were fixed with paraformaldehyde. After blocking with normal goat serum at room temperature for 30 min, the cells were incubated with primary rabbit anti-c-Kit antibody (Abcam, Cambridge, MA, USA) at 4°C overnight, followed by incubation with fluorescein isothiocyanate (FITC)-labelled goat anti-rabbit IgG (Boster). The nuclei were then counterstained with 4',6-di-amidino-2-phenylindole (DAPI), and the coverslips were mounted in an antifade mounting medium (SouthernBiotech, Birmingham, AL,

USA). A fluorescence density analysis and image acquisition were performed using an FV1000 confocal microscope (Olympus, Japan). Intracellular Ca^{2+} concentration was measured using Fluo 3-acetoxymethyl (AM) fluorescence as previously described [20].

Statistical analysis

All data are expressed as means ± standard deviations (SDs). The statistical analyses were performed using SPSS version 18.0 software (SPSS Inc., Chicago, IL, USA). The data were tested for normality and homogeneity of variance. The groups were compared using one-way analysis of variance (ANOVA), followed by a Student-Newman-Keuls (SNK) test (when there was homogeneity of variance between the groups) or Dunnett's T3 test (when the variance was not equal between the groups). *P*-values < 0.05 were considered statistically significant.

Results

Overexpression or downregulation of miR-551b-5p in ICCs

The ICCs cultured *in vitro* were transfected with an miR-551b-5p mimic, an miR-551b-5p inhibitor, or corresponding negative controls. Forty-eight hours after transfection, the expression of miR-551b-5p in the different groups was detected using real-time PCR (**Figure 1**). As expected, miR-551b-5p expression was significantly upregulated in the ICCs transfected with the miR-551b-5p mimic compared to the normal control ICCs or ICCs transfected with the negative control for the miR-551b-5p mimic (*P* < 0.05). In addition, miR-551b-5p expression was significantly downregulated in the ICCs transfected with the miR-551b-5p inhibitor compared to the normal control ICCs or ICCs transfected with the negative control for the miR-551b-5p inhibitor (*P* < 0.05). There were no significant differences in miR-551b-5p expression between the normal control ICCs and ICCs transfected with the negative control for the miR-551b-5p mimic or inhibitor.

Overexpression or downregulation of miR-551b-5p does not influence c-Kit mRNA or protein expression levels

After transfection of the ICCs with the miR-551b-5p mimic or inhibitor, we detected the
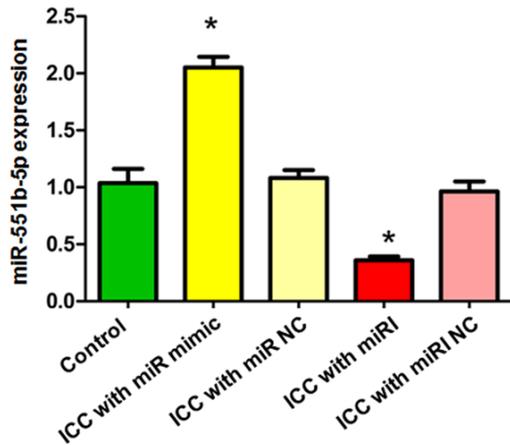
Figure 1. miR-551b-5p expression is promoted by the miR-551b-5p mimic, but attenuated by the miR-551b-5p inhibitor. *P < 0.05 compared to the control group. The data were analyzed using one-way ANOVA, followed by an SNK test. ANOVA: analysis of variance; ICC: interstitial cells of Cajal; miR: miR-551b-5p mimic; miRI: miR-551b-5p inhibitor; NC: negative control; SNK: Student-Newman-Keuls.
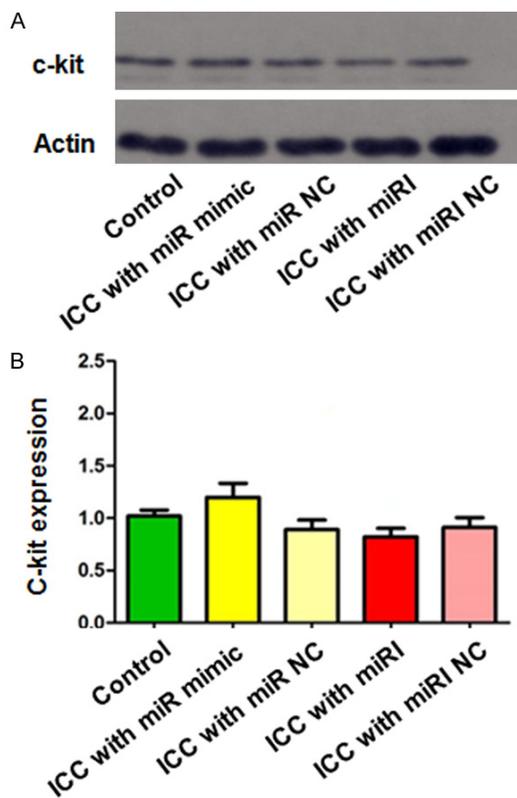
Figure 2. Overexpression or downregulation of miR-551b-5p does not influence c-Kit expression in ICCs. A. Western blot analysis of c-KIT protein in different groups of ICCs; B. Real-time PCR analysis of c-KIT protein expression in different groups of ICCs. The data were analyzed using one-way ANOVA, followed by an SNK test.

c-Kit protein and mRNA expression levels using a Western blot analysis and real-time PCR, respectively. As shown in **Figure 2**, transfection with the miR-551b-5p mimic or inhibitor had no significant effect on c-Kit protein and mRNA expression levels.

Overexpression or downregulation of miR-551b-5p does not influence c-Kit protein localization

Next, we evaluated the influence of transfection with the miR-551b-5p mimic or inhibitor on c-Kit protein expression and localization in ICCs using immunohistochemistry. As shown in **Figure 3**, transfection with the miR-551b-5p mimic or inhibitor had no significant effect on the expression and localization of c-Kit protein in the ICCs.

Overexpression or downregulation of miR-551b-5p significantly alters intracellular Ca^{2+} concentration in ICCs

After transfection of the ICCs with the miR-551b-5p mimic or inhibitor, we determined the intracellular Ca^{2+} concentration using the Fluo 3-AM method. As shown in **Figure 4**, the intracellular Ca^{2+} concentration was significantly increased in cells transfected with the miR-551b-5p mimic compared to the normal control ICCs or ICCs transfected with the negative control for the miR-551b-5p mimic ($P < 0.05$). In addition, the intracellular Ca^{2+} concentration was significantly decreased in the ICCs transfected with the miR-551b-5p inhibitor compared to the normal control ICCs or ICCs transfected with the negative control for the miR-551b-5p inhibitor ($P < 0.05$). There were no significant differences in intracellular Ca^{2+} concentration between the normal control ICCs and ICCs transfected with the negative control for the miR-551b-5p mimic or inhibitor.

Discussion

GID is closely associated with the progression and prognosis of SAP [3]. However, the pathogenesis of GID is still largely unknown, although inflammatory factors [21], nitric oxide [22], and gastrointestinal hormones [3] have been implicated in this process. Because ICCs perform a central role in controlling smooth muscle contraction, which has been implicated in the pathogenesis of SAP-associated GID [10, 12-14], we investigated the role of miR-551b-5p,
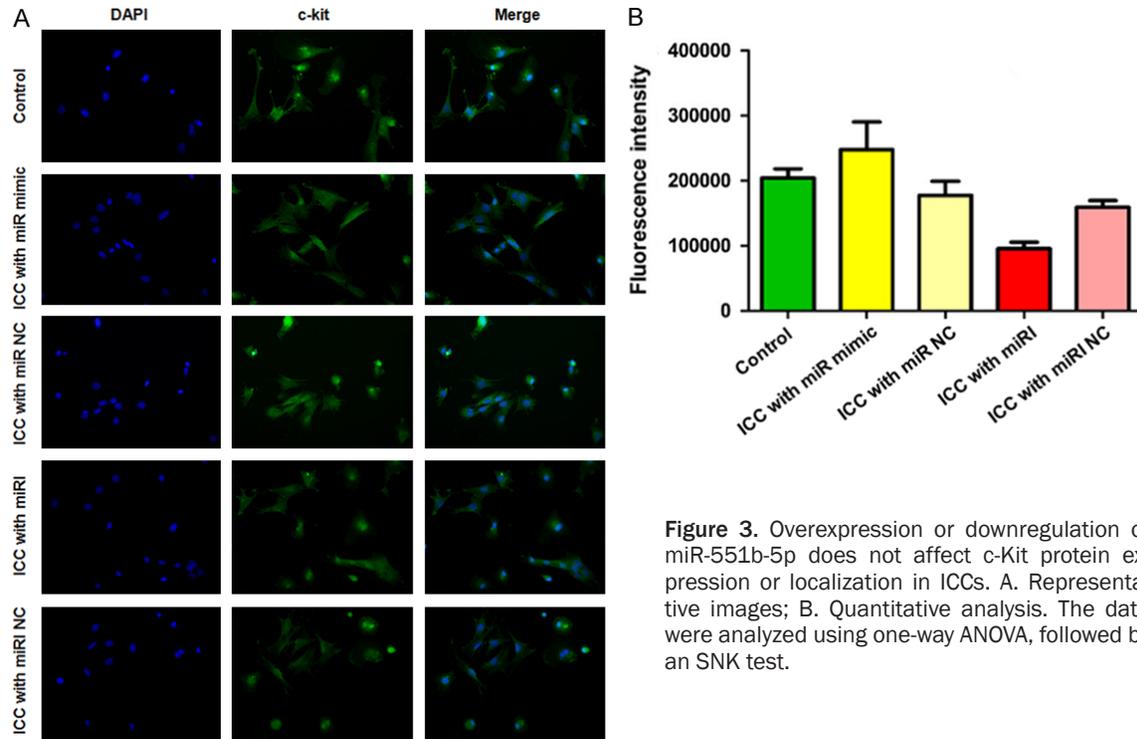
Figure 3. Overexpression or downregulation of miR-551b-5p does not affect c-Kit protein expression or localization in ICCs. A. Representative images; B. Quantitative analysis. The data were analyzed using one-way ANOVA, followed by an SNK test.
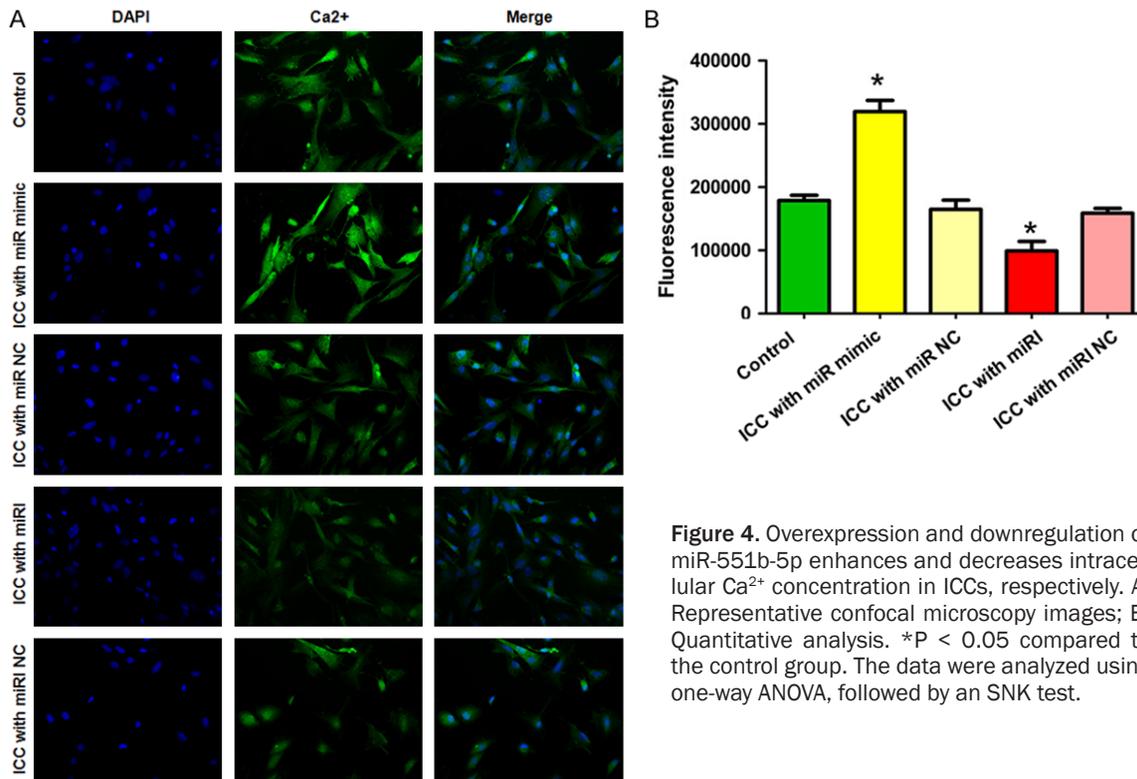
Figure 4. Overexpression and downregulation of miR-551b-5p enhances and decreases intracellular Ca^{2+} concentration in ICCs, respectively. A. Representative confocal microscopy images; B. Quantitative analysis. *P < 0.05 compared to the control group. The data were analyzed using one-way ANOVA, followed by an SNK test.

an miRNA associated with SAP, in ICCs *in vitro*. Unexpectedly, we found that overexpression or downregulation of miR-551b-5p did not significantly alter the expression and distribution of c-Kit, a classic marker for ICCs. However, overexpression of miR-551b-5p significantly

increased the intracellular Ca^{2+} concentration, whereas downregulation of miR-551b-5p significantly decreased it.

It has been well documented that ICCs play a central role in the control of gastrointestinal motility, and loss of or injury to ICCs is associated with a number of pathologies [10, 11]. Damage to ICCs was found to occur in the muscular layer of the small intestine in rats with acute necrotizing pancreatitis (ANP) [14]. In addition, the numbers and ultrastructure of ICCs are clearly altered in SAP rats [12, 13]. Small intestinal paralysis in ANP rats may be related to ICC deficiencies [14]. In a rat model of ANP, octreotide has been shown to ameliorate the severity of ileus, which may be brought about by the minimization of ICC injury [23]. Thus, ICC dysfunction may result in gastrointestinal dysmotility in SAP, and ICCs are a potential target for the treatment of SAP-associated GID.

ICCs in the gastrointestinal tract express c-Kit, and c-Kit signaling is essential for the pacemaker activity of ICCs [15]. Imatinib, a potent inhibitor of c-Kit, blocks spontaneous mechanical activity in the small intestines of adult mice [24]. Mutations of c-Kit in mice have been shown to result in the depletion of ICCs in the gastrointestinal tract [25, 26]. In SAP rats, the numbers and ultrastructure of c-kit-positive cells have been shown to be significantly altered [12-14]. However, we found that overexpression or downregulation of miR-551b-5p did not significantly alter the expression and distribution of c-Kit in ICCs. Because the ICCs used in this study were isolated from normal rats, which, unlike SAP rats, did not have disrupted c-Kit signaling, this finding suggests that miR-551b-5p may act downstream or independently of c-Kit signaling.

Gastrointestinal motility depends on the contractile activity of smooth muscle cells, which is regulated by intracellular Ca^{2+} concentrations [27, 28]. The spontaneous rhythmic contractions and periodic slow waves in gastrointestinal smooth muscles result from ICC pacemaker activity induced by oscillations in Ca^{2+} concentrations [13, 15, 16, 29]. Thus, an abnormal change in intracellular Ca^{2+} concentration may affect the Ca^{2+} concentration oscillation and the ICC pacemaker activity. In the present study, the intracellular Ca^{2+} concentration was significantly increased by overexpression of miR-551b-5p but significantly decreased by downregulation of miR-551b-5p. This suggests that miR-551b-5p may alter the ICC pacemaker activity and spontaneous smooth muscle contraction by regulating intracellular Ca^{2+} concentrations, thereby participating in the pathogenesis of SAP-associated GID. However, the mechanisms by which miR-551b-5p affects intracellular Ca^{2+} concentrations are unknown and elucidating them will require further studies.

Several miRNAs have been implicated in the pathogenesis of GID. Saito et al [30] found that the downregulation of muscle-specific miR-1 and miR-133 is associated with functional gastric emptying in Helicobacter pylori-infected mice. Nezami et al [31] discovered that miR-375 mediates palmitate-induced enteric neuronal damage and high fat diet-induced intestinal transit delay in mice. Moreover, miRNAs that regulate ion channels, transporters, and Ca^{2+} binding proteins in smooth muscles have also been identified. Terentyev et al [32] found that miR-1 enhanced Ca^{2+} release by selectively increasing the phosphorylation of the L-type and ryanodine receptor 2 (RyR2) Ca^{2+} channels. Aurora et al [33] found that miRNA-214 can control Ca^{2+} overload in mice in order to protect the heart from ischemic injury. These findings indicate that miRNAs could play a critical role in the diagnosis and treatment of GID. Our observation that miR-551b-5p can regulate the intracellular Ca^{2+} concentration in ICCs shows that it is a potential target for the treatment of SAP-associated GID.

In conclusion, the present study showed that overexpression of miR-551b-5p significantly increased the intracellular Ca^{2+} concentration in ICCs while downregulation significantly decreased the intracellular Ca^{2+} concentration, suggesting that miR-551b-5p plays a role in the regulation of the ICC pacemaker activity in SAP. However, neither overexpression nor downregulation of miR-551b-5p significantly altered the expression and distribution of c-Kit, suggesting that miR-551b-5p acts downstream or independently of c-Kit signaling. The mechanisms of action of miR-551b-5p in SAP-associated GID require further investigation.

Acknowledgements

Disclosure of conflict of interest

None.

Address correspondence to: Pi Liu, Department of Gastroenterology, The First Affiliated Hospital of Nanchang University, 17 Yongwaizheng Street, Donghu District, Nanchang 330006, Jiangxi Province, China. Tel: +86-791-3969145; E-mail: liupi126@sina.com

References

[1] Banks PA, Bollen TL, Dervenis C, Gooszen HG, Johnson CD, Sarr MG, Tsiotos GG, Vege SS; Acute Pancreatitis Classification Working Group. Classification of acute pancreatitis--2012: revision of the Atlanta classification and definitions by international consensus. Gut 2013; 62: 102-111.

[2] Petrov MS, Shanbhag S, Chakraborty M, Phillips AR and Windsor JA. Organ failure and infection of pancreatic necrosis as determinants of mortality in patients with acute pancreatitis. Gastroenterology 2010; 139: 813-820.

[3] Wang X, Gong Z, Wu K, Wang B and Yuang Y. Gastrointestinal dysmotility in patients with acute pancreatitis. J Gastroenterol Hepatol 2003; 18: 57-62.

[4] Bhatia M and Moochhala S. Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. J Pathol 2004; 202: 145-156.

[5] Seerden TC, De Man JG, Holzer P, Van den Bossche RM, Herman AG, Pelckmans PA and De Winter BY. Experimental pancreatitis disturbs gastrointestinal and colonic motility in mice: effect of the prokinetic agent tegaserod. Neurogastroenterol Motil 2007; 19: 856-864.

[6] Van Felius ID, Akkermans LM, Bosscha K, Verheem A, Harmsen W, Visser MR and Gooszen HG. Interdigestive small bowel motility and duodenal bacterial overgrowth in experimental acute pancreatitis. Neurogastroenterol Motil 2003; 15: 267-276.

[7] Ammori BJ. Role of the gut in the course of severe acute pancreatitis. Pancreas 2003; 26: 122-129.

[8] Koh YY, Jeon WK, Cho YK, Kim HJ, Chung WG, Chon CU, Oh TY and Shin JH. The effect of intestinal permeability and endotoxemia on the prognosis of acute pancreatitis. Gut Liver 2012; 6: 505-511.

[9] Tian R, Tan JT, Wang RL, Xie H, Qian YB and Yu KL. The role of intestinal mucosa oxidative stress in gut barrier dysfunction of severe acute pancreatitis. Eur Rev Med Pharmacol Sci 2013; 17: 349-355.

[10] Sanders KM and Ward SM. Interstitial cells of Cajal: a new perspective on smooth muscle function. J Physiol 2006; 576: 721-726.

[11] Huizinga JD and Chen JH. Interstitial cells of Cajal: update on basic and clinical science. Curr Gastroenterol Rep 2014; 16: 363.

[12] Shi LL, Liu MD, Chen M and Zou XP. Involvement of interstitial cells of Cajal in experimental severe acute pancreatitis in rats. World J Gastroenterol 2013; 19: 2179-2186.

[13] Zhao Y, Li G, Sun X, Xu P, Lou X and Tang M. Changes of amount and ultrastructure of interstitial cells of Cajal in rats with severe acute pancreatitis and their importance. Int J Clin Exp Med 2015; 8: 5492-5498.

[14] Zhou H, Liu L, Bai Y, Wu W, Li G, Li J, Zou D, Gao J and Li Z. Damage of the interstitial cells of Cajal and myenteric neurons causing ileus in acute necrotizing pancreatitis rats. Surgery 2011; 149: 262-275.

[15] Lee HT, Hennig GW, Park KJ, Bayguinov PO, Ward SM, Sanders KM and Smith TK. Heterogeneities in ICC Ca2+ activity within canine large intestine. Gastroenterology 2009; 136: 2226-2236.

[16] Ward SM, Ordog T, Koh SD, Baker SA, Jun JY, Amberg G, Monaghan K and Sanders KM. Pacemaking in interstitial cells of Cajal depends upon calcium handling by endoplasmic reticulum and mitochondria. J Physiol 2000; 525 Pt 2: 355-361.

[17] Liu P, Xia L, Zhang WL, Ke HJ, Su T, Deng LB, Chen YX and Lv NH. Identification of serum microRNAs as diagnostic and prognostic biomarkers for acute pancreatitis. Pancreatology 2014; 14: 159-166.

[18] Malysz J, Gibbons SJ, Saravanaperumal SA, Du P, Eisenman ST, Cao C, Oh U, Saur D, Klein S, Ordog T and Farrugia G. Conditional genetic deletion of Ano1 in interstitial cells of Cajal impairs Ca2+ transients and slow-waves in adult mouse small intestine. Am J Physiol Gastrointest Liver Physiol 2017; 312: G228-G245.

[19] Zhu G, Li J, He L, Wang X and Hong X. MPTP-induced changes in hippocampal synaptic plasticity and memory are prevented by memantine through the BDNF-TrkB pathway. Br J Pharmacol 2015; 172: 2354-2368.

[20] Rijkers GT, Justement LB, Griffioen AW and Cambier JC. Improved method for measuring intracellular Ca++ with fluo-3. Cytometry 1990; 11: 923-927.

[21] Natale L, Piepoli AL, De Salvia MA, De Salvatore G, Mitolo CI, Marzullo A, Portincasa P, Moschetta A, Palasciano G and Mitolo-Chieppa D. Interleukins 1 beta and 6 induce functional alteration of rat colonic motility: an in vitro study. Eur J Clin Invest 2003; 33: 704-712.

[22] Kuiken SD, Tytgat GN and Boeckxstaens GE. Role of endogenous nitric oxide in regulating antropyloroduodenal motility in humans. Am J Gastroenterol 2002; 97: 1661-1667.

[23] Zhou H, Gao J, Wu W, Liu L, Wei L, Zou D and Li Z. Octreotide ameliorates intestinal dysmotility by interstitial cells of Cajal protection in a rat acute necrotizing pancreatitis model. Pancreas 2011; 40: 1226-1233.

[24] Shimojima N, Nakaki T, Morikawa Y, Hoshino K and Kitajima M. Imatinib blocks spontaneous mechanical activities in the adult mouse small intestine: possible inhibition of c-Kit signaling. Pharmacology 2005; 74: 95-99.

[25] Hirota S, Isozaki K, Nishida T and Kitamura Y. Effects of loss-of-function and gain-of-function mutations of c-kit on the gastrointestinal tract. J Gastroenterol 2000; 35 Suppl 12: 75-79.

[26] Kitamura Y, Hirota S and Nishida T. A loss-of-function mutation of c-kit results in depletion of mast cells and interstitial cells of Cajal, while its gain-of-function mutation results in their oncogenesis. Mutat Res 2001; 477: 165-171.

[27] Perrino BA. Regulation of gastrointestinal motility by Ca2+/calmodulin-stimulated protein kinase II. Arch Biochem Biophys 2011; 510: 174-181.

[28] Sanders KM, Koh SD, Ro S and Ward SM. Regulation of gastrointestinal motility--insights from smooth muscle biology. Nat Rev Gastroenterol Hepatol 2012; 9: 633-645.

[29] Malysz J, Donnelly G and Huizinga JD. Regulation of slow wave frequency by IP(3)-sensitive calcium release in the murine small intestine. Am J Physiol Gastrointest Liver Physiol 2001; 280: G439-448.

[30] Saito Y, Suzuki H, Tsugawa H, Suzuki S, Matsuzaki J, Hirata K and Hibi T. Dysfunctional gastric emptying with down-regulation of muscle-specific microRNAs in Helicobacter pylori-infected mice. Gastroenterology 2011; 140: 189-198.

[31] Nezami BG, Mwangi SM, Lee JE, Jeppsson S, Anitha M, Yarandi SS, Farris AB 3rd and Srinivasan S. MicroRNA 375 mediates palmitate-induced enteric neuronal damage and high-fat diet-induced delayed intestinal transit in mice. Gastroenterology 2014; 146: 473-483, e473.

[32] Terentyev D, Belevych AE, Terentyeva R, Martin MM, Malana GE, Kuhn DE, Abdellatif M, Feldman DS, Elton TS and Gyorke S. miR-1 overexpression enhances Ca(2+) release and promotes cardiac arrhythmogenesis by targeting PP2A regulatory subunit B56alpha and causing CaMKII-dependent hyperphosphorylation of RyR2. Circ Res 2009; 104: 514-521.

[33] Aurora AB, Mahmoud AI, Luo X, Johnson BA, van Rooij E, Matsuzaki S, Humphries KM, Hill JA, Bassel-Duby R, Sadek HA and Olson EN. MicroRNA-214 protects the mouse heart from ischemic injury by controlling Ca(2)(+) overload and cell death. J Clin Invest 2012; 122: 1222-1232.