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**Role of mast cell-miR-490-5p in irritable bowel syndrome**

Ren HX *et al.* miR-490-5p with IBS

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**Abstract**

***AIM***

To explore the functional role of miR-490-5p in mast cell proliferation and apoptosis and in mast cell tryptase/PAR-2 signal pathway.

***METHODS***

The 3rd generation of lentivirus vector systems containing enhanced green fluorescent protein (EGFP) (Ruisai Inc, Shanghai, China), which act as a reporter gene was used to construct the mmu-miR-490-5p lentivirus expression vector pEGFP-antagomiR-490-5p,and the lentivirus vector pEGFP-negative was used as a negative control. And then, the stable transfected mast cell p815 was constructed. GFP positive cells were successfully transfected cells. We determined the expression of miR-490-5p in mast cell p815 before and after transfection using quantitative real-time PCR (qRT-PCR). Additionally, after transduction with the lentivirus vectors, the role of miR-490-5p in mast cell proliferation and apoptosis was investigated by the cck-8 assay and flow cytometry, respectively, the mRNA levels of tryptase and PAR-2 were detected by qRT-PCR and the protein levels were detected by western blot.

***RESULTS***

The inhibition of miR-490-5p expression could promote apoptosis and inhibit proliferation of mast cell p815. The mRNA levels of tryptase and PAR-2 were significantly increased after transfection compared with the control group, tryptase (*P* = 0.721 normal *vs* Null, *P* =0.001 siRNA vs normal, *P* = 0.002 siRNA *vs* null) and PAR-2 (*P* = 0.027, siRNA *vs* null; *P* = 0.353, normal *vs* null; *P* = 0.105, siRAN *vs* normal). The protein levels of tryptase and PAR2 were slightly higher in the siRNA group than that in the control group，but there was no statistical significance(*P* > 0.05).

***CONCLUSION***

miR-490-5p plays a vital role in the pathogenesis of irritable bowel syndrome through affecting the mast cell proliferation and apoptosis; with the down regulation of miR-490-5p ,the mRNA level of mast cell tryptase and PAR-2 increased ,the protein level increased but not statistically significant.

**Key words:** miR-490-5p; Mast cell tryptase; PAR-2; Irritable bowel syndrome

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**Core tip:** Mast cells and mast cell degranulation played important role in the pathophysiology of irritable bowel syndrome .miRNA as a class of important endogenous single strand non coding RNA , plays an essential regulatory role in complex biological systems without protein translation. In our study we aimed to detect the role of miR-490-5p in regulating mast cell proliferation and apoptosis and the expression of mast cell tryptase and PAR-2. Thus, we can predict the role of miR-490-5p in the pathogenesis of irritable bowel syndrome.

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**INTRODUCTION**

Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal (GI) disorders. The cause of IBS remains unknown and seems to be multifactorial[1-4]. Several mechanisms including visceral hypersensitivity, motility disorder ,infection, and psychiatric factors, have been suggested as possible etiologic links to the development of IBS[5,6].Additionally, there has been increasing evidence of a genetic contribution in IBS such as gene polymorphism[7-9] and dysregulated microRNA expression[10-14]. Mast cells and mast cell degranulation function as a bridge in the neuro-immuno-endocrine system in IBS[15]. Tryptase is one of the most important proteins in mast cell degranulation and it exerts its effects mainly through tryptase-PAR-2 signal pathway[16]. However, considering the complexity of the expression and function of tryptase, there are obvious limitations in the single analysis of the signal pathway. Multiple factors are involved in the regulation of the above signaling pathway. Among them, we note that, intracellular concentration of Ca2+, calmodulin ,DAG/PKC and Rho GTPase will affect mast cell degranulation[17,18]. miRNA is a class of important endogenous single strand non coding RNA , plays an essential regulatory role in complex biological systems without protein translation. Mature miRNA down regulates gene expression by binding to the 3′-UTR region of the target gene and cause translational inhibition or mRNA degradation ultimately moderating the protein expression level. To date, multiple dysregulated microRNA expression had been reported in IBS. For example, miRNA-29a affects intestinal membrane permeability through its regulation of glutamate-ammonia ligase gene and miRNA-510 play an important role in the regulation of 5-HT3E expression[19,20].previously, we had detected an elevation of miR-490-5p in IBS-D patients by the high-throughput microarray ,and it had not been researched in IBS. Target Scan was used to predict the target gene of miR-490-5p, we noted that: *CABP5*: encoding calcium binding protein5;

*CAMK1D*: encoding calcium/calmodulin-dependent protein kinase ID; *CASP3*: encoding caspase 3, apoptosis-related cysteine peptidase; TNFSF18: encoding tumor necrosis factor (ligand) superfamily, member 18; *BOP1*: encoding proliferation-associated protein. Among them both calcium and calmodulin-dependent protein kinase were highly correlated with mast cell degranulation[17,18]. Caspase, apoptosis-related cysteine peptidase and proliferation associated protein still have been reported to be involved in cell proliferation and apoptosis in other disease[21]. However, no study is available, concerning the relationship between them in IBS. By comprehensively analyzing the results of gene microarray and bioinformatics analysis , we focused our studies on the expression and functional role of miR-490-5P in mast cells and mast cell/tryptase/PAR-2signaling pathway, and then to predict whether and how miR-490-5P was involved in the pathogenesis of IBS. Maybe our study may provide a theoretical basis for further study of biomarkers for the diagnosis and new treatment of IBS.

**MATERIALS AND METHODS**

***Cell line***

Mast cell p815was purchased Shanghai Cell Biochemical Institute, China Academy of Science (Shanghai, China). This cell line is a suitable transfection host.P815 cells phagocytose latex beads but not zymosan or BCG. They do not function in antibody- dependent cell- mediated cytotoxicity. The growth of the cells is not inhibited by dextran sulfate, LPS or PPD. The cells were also found to be negative for the ectromelia virus (mousepox).

***Reagents***

Mast cells were cultured in DMEM culture medium with 10% fetal bovine serum (FBS), PBS, trypsin, penicillin streptomycin combination, miR-490-5p-siRNA vector, siRNA negative lentiviral vector, blasticidin, RIPA lysate (50 mmol/L Tris [pH 7.4], 150 mmol/L NaCl, 5 mmol/L EDTA, 1% Triton X-100 [v/v]), protease inhibitor cocktail,(1 mmol/L sodium vanadate, and 10 mmol/L NaF). DNA marker, agarose, EB substitute. Trizol and cDNA reverse transcription kit were obtained from Takara. Polymerase chain reaction (PCR) primers were synthesized by Sangon Biotechnology (Shanghai, China). SuperReal PreMix Plus was purchased from Tiangen Biotechnology (Beijing, China).Mouse anti-GAPDH monoclonal antibody for western blot was obtained from Cell Signaling Technology. Anti-PAR-2 monoclonal antibody for western blot was obtained from abcam. Antitryptase clonal antibody for western blot was purchased from Santa Cruz Biotechnology.

***Screening for target miRNA***

Based on the results of a previous high-throughput microarray and bioinformatics analysis, TargetScan analysis (http://www.targetscan.org) was used to predict the target gene of the miRNA. Subsequently, a GO enrichment analysis and a KEGG pathway analysis of the target gene were performed. Based on the results of the bioinformatics analysis, we searched miRDB and miRBase to query the specific features of target genes of interest. Our selection criteria for the target miRNA were as follows: (1) it is abnormally expressed in IBS-D patients[22]; (2) the biological function of its target gene should related to mast cell degranulation, proliferation and apoptosis or signal transduction pathway or visceral sensitivity or neurotransmitter -release associated protein or ntracellular material transportation-associated protein, because the products of these target gene may highly correlated with the pathogenesis of IBS[23]; and (3) it exists both in human and mouse mast cells. Finally, we choose miR-490-5P as our target miRNA.

***miR-490-5p RNA interference in mast cells***

The 3rd generation of lentivirus vector system containing an expression vector and 3 packaging auxiliary plasmids was used to construct the miR-490-5p recombinant silencing vector. The expression vector containing the basic components of HIV 5 'LTR and 3' LTR as well as Other auxiliary components, CMV promoter and Blasticidin markers and enhanced green fluorescent protein(EGFP). Packaging auxiliary plasmid containing pGag-Pol, PRev and pVSVG. According to the mouse mmu-miR-490-5p (MIMAT0017261) gene sequence in the miBase database (5’- CCAUGGAUCUCCAGGUGGGU -3’), a pair of oligonucleotide chains containing the miR-490-5p sequence and the reverse complementary miR-145 sequence, which can form an shRNA precursor sequence containing a stem-loop structure after annealing, were designed. (Top Strand: 5'- TGCTGACCCACCTGGAGATCCATGGGTTTTGGCCACTGACTGACCCATGGATCCAGGTGGGT -3'Bottom Strand:5'- CCTGACCCACCTGGATCCATGGGTCAGTCAGTGGCCAAAACCCATGGATCTCCAGGTGGGTC -3').Then, The shRNA was connected with the linearized miRNA vector, the product was used to transform the Escherichia coli DH5α,the recombinant plasmids were extracted and analyzed by sequencing , named pcDNA6.2-EGFP-mmu-490-5p.Then,using pcDNA6.2-EGFP-mmu-490-5p as template to amplify. Primers：

(Lenti-Asc1-F:5'-TACTGGCGCGCCGCCACCATGGTGAGCAAGGGCGAGGA-3';

Lenti-Pme1-R：5'-ACTAGTTTAAACTGCGGCCAGATCTGGGC-3')

The pLV-shRNA lentiviral expression vector was generated via T4 DNA ligase, and an unrelated negative control sequence was established in the same manner. The above protocol was completed by Shanghai R&S Biotechnology Co., Ltd (Shanghai, China).

HEK-293T cells (5 × 104; ATCC, Maryland, United States) were seeded in a 6-well cell culture plate in Dulbecco's modified Eagle medium (DMEM) and incubated at 37 °C with 5% CO2 for 24 h. When the cells reached 50%-70% confluence, pLV-shRNA plasmids and auxiliary plasmids were co transfected into them using Lipo3000. After 48 h of transfection, the cell supernatant was collected for titer determination. Gradient dilution was performed for the supernatant using phosphate-buffered saline (PBS) at ratios of 10−1-10−6. Three wells were used for each gradient, and 50 μL of lentiviral diluent was placed in each well for infection. After 48 h of infection, we recorded the number of infected fluorescent cells at the dilution gradient where the ratio of green fluorescent protein (GFP)+cells was approximately 20%, and mean values were calculated. Lentiviral titers were calculated according to the following formula (BT = TU/ml): TU/uL = (P × N/100 × V) × 1/DF (P = number of GFP+ cells, N = 105, V = volume of lentiviral diluent = 50 μL, DF = dilution factor

***Construction of the stably transfected cell line***

Well-growing mast cells were seeded in 24-well plates (30000 cells per well). After adherence and the cells density reached 50%, the cells were transduced with recombinant lentivirus vectors at a multiplicity of infection (MOI) of 60. The cells were maintained at 37 °C in a humidified atmosphere of 5% CO2 for 24 h, and then the medium was changed normally. After 48 h of transfection, the transfection efficiency was assessed by nversion fluorescence microscopy. thereafter, Thereafter, the positively transfected cells were selected by applying blasticidin in the medium at the minimal effective dose of 6 μg/mL (the minimal effective dose was defined as the dose at which most of the cells died within 7 d).The efficiency of GFP expression was detected by flow cytometry, when the expression efficiency of GFP was more than 85%，the dose of blasticidin was replaced by half of the minimal effective dose.

***Detection of the miR-490-5p level after silencing***

Total RNA was extracted both from both normal cells and the stably transfected cells using Trizol (Roche, Mannheim, Germany) according to the manufacturer’s instructions. Complementary DNA (cDNA) templates were synthesized using the PrimeScriptTM RT reagent Kit (Takara, Danian, China) after the concentration and purity of the total RNA were measured by Nano drop. The expression level of miR-490-5p was measured by qRT-PCR, The primers used in RT-PCR were listed in Table 1.

***Cell proliferation assay***

The cell counting kit-8 (cck-8) assay was used to examine the cell proliferation. First, we constructed a standard curve. Next, the cells were washed three times with phosphate-buffered saline (PBS), resuspended in PBS, counted, and diluted 1:2 geometrically. The cells were seeded in 96-well plates at different final concentrations (3 × 104, 6 × 104, 12 × 104, 24 × 104, 48 × 104 per ml) in assay medium and incubated at 37°C in a 5% CO2 incubator for approximately 2-4 h. Next, 10 μL of cck-8 was added to each well, and the cells were incubated at 37 °C. At different time points (1 h, 2 h, 3 h, 4 h), the absorbance at 490 nm was read on a GF-M3000 microplate reader (Gaomicaihong Analysis Instrument Company, Shandong, China). The absorbance was used as the Y-axis, and the cell number was used as the X-axis by drawinga standard curve to determine the optimal number of cells and the time of detection. Additionally, using the standard curve, we could determine whether CCK-8 is suitable for determining the level of proliferation of mast cells. In the second step, we washed, resuspended, and counted the cells as described above. They were then seeded in 96-well plates at a final concentration of 3 × 104 per mL in assay medium and incubated at 37 °C in a 5% CO2 incubator. At different time points (24 h, 48 h, 72 h), 10 μL of cck-8 was added to each well, and the cells were incubated at 37 °C for another 4 h. The absorbance at 490 nm was read on a microplate reader.

***Annexin V-PE/7-AAD double-staining flow cytometry for the detection of apoptosis***

Cell apoptosis was detected by flow cytometry (FCM) and analyzed by CellQuest software (Becton Dickinson, Bedford, MA).The transfected cells and normal cells were collected, washed three times with cool PBS, and then resuspended in PBS, next 1 × 106cells were processed forlabeling with Annexin V/7AAD according to the PE Annexin V apoptosis detection kit (BD Biosciences, Franklin Lakes, NJ, United States).

***Real-time polymerase chain reaction***

Real- time PCR was used to detect the mRNA level of tryptase and PAR-2 in mast cell p815 after transfection. Total RNA was extracted from both from normal cells and the stably transfected cells using Trizol .The total RNA was transcribed using the reverse transcription kit after measuring concentration of total RNA with Nano drop. Total RNA was quantified to 1 μg. The information regarding the primers of tryptase, PAR-2 and U6 is listed in Table 2. The PCR samples were set up in a volume of 20 μL, containing 2 μL of the cDNA diluted 1:5 with PCR grade water, 10.4 μL of SYBR Green Supermix, and 300 nmol/L of the forward and reverse primer each. The PCR conditions consisted of preliminary denaturation at 95 ℃ for 15 min, followed by 40 cycles of 95 ℃ for 10 s, 60 ℃ for 32 s. Melting curve analysis was performed to confirm the specificity as well as the integrity of the PCR products by the presence of a single peak. Products were subjected to agarose gel electrophoresis. Expression levels of the mRNA were quantified by calculating threshold cycle values compare with the u6 endogenous control using 2-DDCt.

***Western blot***

Total protein was extracted from mast cell P815 and lysed in RIPA lysate (50 mmol/L Tris [pH 7.4], 150 mmol/L NaCl, 5 mmol/L EDTA, 1% Triton X-100 [v/v], 1 × protease inhibitor cocktail, 1 mM sodium vanadate, and 10 mmol/L NaF). Equal amounts of total protein were separated by SDS-PAGE with a 12% resolving layer and a 4% stacking layer and the proteins were transferred to PVDF membranes at 300 mmA for 1.5 hour. After blocking in 5% nonfat milk diluted with TBS-T for 1 h at room temperature with shaking, the membranes were incubated with primary antibodies (working dilution of tryptase 1:300; PAR-2 1:400) with gentle shaking overnight at 4 ℃, washed with TBST（3 times for 5 min each）and then incubated with species-appropriate secondary antibodies for 2 hours at 37 ℃. After washing in TBS-T (3 times for 5 min each), membranes were incubated, using the ECL Developer Kit in a dark room. The efficiency of protein loading and transfer was assessed by reprobing the membranes with an anti-GAPDH antibody. The density of each band was analyzed using the Image J software. The expression of the target protein band was compared with that of the corresponding control band.

***Statistical analysis***

The experimental data are represented as the means±standard deviation. SPSS 20.0 was used for data analysis. One-way analysis of variance was used for analysis. Spearman analysis was used for correlation analysis. A p value less than 0.05 was considered to indicate statistical significance.

**RESULTS**

***Transfection efficiency and miR-490-5p level after silencing***

The tansfection rate was measured by flow cytometry and the result showed that the efficiency was more than 85% in both siRNA group and null group.We performed qRT-PCR to confirm the expression of miR-490-5p in mast cell p815 after silencing. As shown in Figure 1 ,the level of miR-490-5p in the siRNA group was significantly decreased compared with the empty vector group(*P* < 0.05).

***Proliferation and apoptosis***

From the results of the standard curve，we found that when the number of varied between 3000 and 48000，there was a linear correlation between the number of cells and OD value .Therefore, CCK-8 was suitable for determining the level of proliferation of mast cells .In the proliferation test, the results showed that inhibition of miR-490-5P expression could significantly reduce the cell viability compared with the control group (*P* < 0.05).In the apoptosis test, the apoptosis rate was significantly higher following the inhibition of miR-490-5p expression than the control group (*P* < 0.05), as shown in Figure 2.

***mRNA levels of tryptase and PAR-2***

The mRNA levels of tryptase (*P* = 0.721 normal *vs* null, *P* = 0.001 siRNA *vs* normal *P* = 0.002 siRNA *vs* null) and PAR-2 (*P* = 0.027 siRNA *vs* null, *P* = 0.353 normal *vs* null, *P* = 0.105 siRAN *vs* normal) were higher in the siRNA group than in the normal and null groups. Additionally, the difference between the groups was statistically significant, and all of the real-time PCR products from the three groups migrated as expected (Figure 3 and 4).

***Protein levels of tryptase and PAR-2***

The expression results of siRNA group were compared with normal and null controls. The density of each band was compared with the corresponding control band and normalized to the GAPDH gene .Elevated expression of tryptase and PAR-2 was found in the siRNA group compared with both normal and null group, but there were no significant differences between the siRNA group and the controls (*P* > 0.05) (Figure 5).

**DISCUSSION**

In our study, we found that miR-490-5p promotes mast cell proliferation and apoptosis resistance. The inhibition of miR-490-5P expression could significantly increase the mRNA level of mast cell tryptase and PAR2，but the protein level of tryptase and PAR2 increased slightly ,and we did not observe a statistically significant difference between the siRNA and the control groups.

miRNAs are an abundant class of 20-22-nt noncoding single-strand RNA and play significant roles in various physiological and pathological processes[24,25]. Many miRNAs have been shown to be associated with IBS. Previously we had detected an elevation of miR-490-5p in IBS-D patients. miR-490-5p is one of members of miR-490 family. The expression and functional role of miR-490-5p had also been reported in other disease. It was involved in cell proliferation, apoptosis and the regulation of signaling pathways through different ways. In the study of Shiqi Li, miR-490-5p was found to be a novel tumor suppressor of bladder cancer cell proliferation through targeting c-Fos[26,27]. In a test of renal cancer miR-490-5p was verified to directly bind to 3’UTR of the PIK3CA mRNA and reduce the expression of PIK3CA at both mRNA and protein levels, which further inhibits phosphatidylinositol 3-kinase/Akt signalling pathway[28]. While, the role of miRNA-490-5p in the development and progression of IBS had not been reported and its target gene such as *CABP5*: encoding calcium binding protein5; *CAMK1D*: encoding calcium/calmodulin-dependent protein kinase ID; *CASP3*: encoding caspase 3, apoptosis-related cysteine peptidase; TNFSF18: encoding tumor necrosis factor (ligand) superfamily, member 18; *BOP1*:encoding proliferation-associated protein. Among them both calcium and calmodulin-dependent protein kinase were highly correlated with mast cell degranulation[17,18]. Caspase, apoptosis-related cysteine peptidase and proliferation associated protein still have been reported to be involved in cell proliferation and apoptosis. In our study, we found that inhibition of miR-490-5p may significantly reduce mast cell proliferation and promote apoptosis. What is more the level of miR-490-5p is highly correlated with the mRNA level of mast cell tryptase and PAR2, however, why the elevation of PAR-2 and tryptase mRNA did not lead to a significant elevation of PAR-protein and tryptase-protein remains unknown. Possible reasons were analyzed as follows: Firstly, some other factors may be involved in the post-transcriptional regulation or the protein translation process. Secondly, the role of miR-490-5P is mainly reflected in up-regulating the function of tryptase/PAR-2 rather than by affecting the expression of them. The third, the mast cells were merely normal cells in the resting state, lacking activating factors; however, in IBS-D patients, the mast cells were activated. We were the first time to detect the role of miR-490-5p in IBS, and the information about the biological role of miRNA in the IBS which could be used for reference was not much. While, previous studies had reported that the number of intestinal mucosal mast cells increased in IBS patients and the mast cell tryptase/ PAR-2 signal pathway played an important role in the pathogenesis of IBS .So any factors which associated with the above process should arouse our attention. In summary, although there were some problems to be further studied in our research, we could still conclude that the mast cell miR-490-5p may participate in the occurrence and development of IBS through regulating the proliferation and apoptosis of mast cells, but its effect in mast cell tryptase/PAR-2 signal pathway was intricate. The inadequacies of this study was that we just found that mir-490-5p is highly correlated with mast cell proliferation and apoptosis, but the specific target gene through which miR-490-5p play a crucial role in IBS still remains unknown, what is more, as for the elevation of tryptase and PAR-2 mRNA did not lead to an increasing of protein level, yet to be explored. The next step, we should screen out the target gene of miR-490-5P which involved in the regulation of the above biological process by bioinformatics analysis, knockout the target gene selectively, and then, to redetect the level of mast cell tryptase and PAR2.maybe this will provide a new target for the treatment of IBS.

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**COMMENTS**

***Background***

Previous studies have reported that mast cells and mast cell degranulation were involved in the pathogenesis of irritable bowel syndrome (IBS). Tryptase was an important component of the mast cell degranulation which lead to visceral hypersensitivity of IBS patients by activating PAR-2. The authors previous studies had detected that IBS patients existed the dysregulation of mir-490-5p whose target gene was highly correlated with visceral sensitivity or neurotransmitter release associated protein, or intracellular material transportation associated protein. So we predicted that miR-490-5p may indirectly involved in the regulation of mast cell-tryptase-par-2 signaling pathway.

***Research frontiers***

There are a growing number of researches report that different miRNAs are involvedin the Regulation of proliferation and differentiation of epithelial cells and smooth muscle cells of digestive tract. The latest research reported that MiRNA plays an important role in the differentiation, proliferation and functional regulation of bone marrow mast cell (BMMCs) cells in the inflammatory response. Another research reported that miR-29a was highly correlated with the increase of intestinal permeability in IBS-D patients. Thus we can conclude that the regulation of miRNA may be closely related to the pathogenesis of IBS.

***Innovations and breakthroughs***

We proved for the first time that miR-490-5p may directly or indirectly involved in mast cell Proliferation and apoptosis and mast cell/ tryptase/PAR-2 signaling pathway which paly important role in IBS-D.

***Applications***

miRNA-490-5p may become a new biomarker for diagnosis of IBS and new target for therapy.

***Terminology***

RNA interference (RNAi), an absolutely fundamental biological process by which cells regulate gene expression, acts through complementary base-paring with target mRNA and retrieves cellular RNases which in turn degrade mRNA transcripts. RNAi is now routinely used to evaluate gene function both in vitro and in vivo and many innovative screens reported the use of RNAi to investigate potential drug targets

***Peer-review***

The author supported that miR-490-5p may directly or indirectly involved in the pathogenesis of IBS. It was the first research focusing on the miR-490-5p and IBS and it really could improve our understanding of IBS to some extent.

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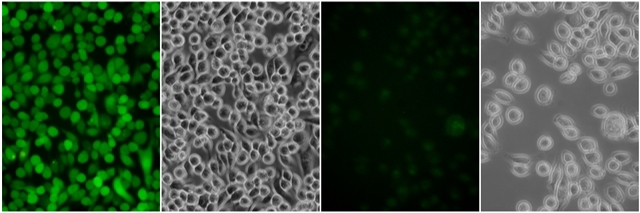
Grade E (Poor): 0

**Table 1 Primers for mmu-miR-490-5p and U6**

|  |  |  |
| --- | --- | --- |
| mmu-miR-490-5p | Forward | TGG CGG CCA TGG ATC TCC AG |
| RT | GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACCCAC |
| Reverse | ATCCAGTGCAGGGTCCGAGG |
| U6 | Forward | CGCTTCACGAATTTGCGTGTGTCAT |
| Reverse | GCTTCGGCAGCACATATACTAAAAT |

**Table 2 Primers for real-time polymerase chain reaction**

|  |  |  |  |
| --- | --- | --- | --- |
| Gene | Forword/reverse primer | PCR product, bp | gene bank NO. |
| tryptase | GCCTCTCCCACCTCCTTATC/GGTATTTCCAGCACACAGCA | 145 | NM\_010781.3 |
| PAR-2 | AGTTCTCTGCGTCCATCCTC/GGGTGTTTCTTCTTCGTTCG | 139 | NM\_007974.4 |
| U6 | CGCTTCACGAATTTGCGTGTGTCAT/ATCCAGTGCAGGGTCCGAGG | 106 | NM-0012042741 |

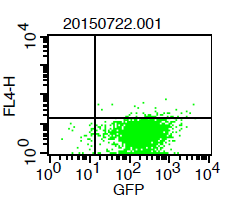
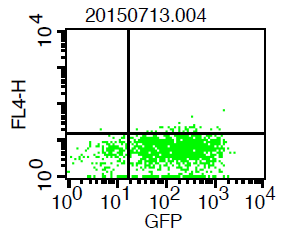


siRNA siRNA Null Null

A

****

B



C

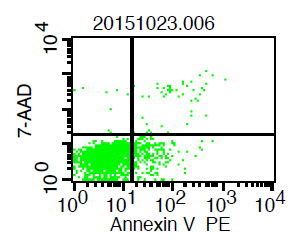
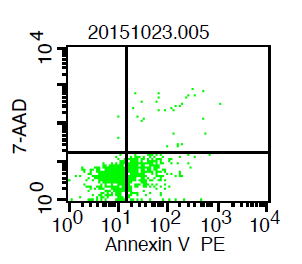
SiRNA Null

**Figure 1 Transduction efficiency and the expression of miR-490-5P after transduction.** A: Renderings of mast cells after lentivirus transfection, the transduction rate of siRNA group was nearly 96％; the transduction rate of the null group was nearly 86％; B: The relative expression of miR-490-5P after transduction, it was significantly decreased in siRNA group compared with the null group; C: Mast cell transfection efficiency detected by flow cytometry, the efficiency of each group was more than 85％.



A

Standard curve Multiplication curve



B

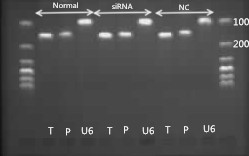
Normal siRNA Null

**Figure 2** **Effect of miR-490-5p on proliferation and apoptosis of mast cells p815.** A: Effect of miR-490-5P on Proliferation of mast cell p815, the cell viability of siRNA group was significantly reduced compared to the normal and null group (*P*＜0.05)，there was no significant difference between normal and null group; B: Mast cell apoptosis after transfection ,in normal group ,the mean apoptotic index was 15.86％, in siRNA group, the mean apoptotic index was 41.19％, in null group, the mean apoptotic index was 11.84％(*n* = 3).



Tryptase PAR-2

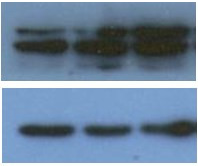
**Figure 3 Quantification of tryptase and protease-activated receptor 2 mRNA after normalization to the housekeeping gene U6.** Significant elevation of tryptase and PAR-2 expression was observed in siRNA group compared with the normal and null group (*P*＜0.05), and no difference was observed between normal and null group. The fold change in mRNA expression was calculated relative to the endogenous U6 control using 2-ΔΔCt. The data are expressed as the mean ± SEM.



**Figure 4 Polymerase chain reaction products were subjected to agarose gel electrophoresis.** All the real-time-PCR products from the three groups migrated as expected. T: Tryptase 145 bp; P: Protease-activated receptor 2; 139 bp; U6: 106bp.



PAR-2 Tryptase



PAR2

Tryptase

GAPDH

GAPDH

**Figure 5 Expression of tryptase and protease-activated receptor 2 in the mast cell p815 after silencing miR-490-5P.** The density of each group band was compared with the corresponding control band and normalized to the *GAPDH* gene. The level of PAR-2 increased in the siRNA group, but no significant difference was observed between siRNA group and the controls (*P*＞0.05). The level of tryptase slightly increased in the siRNA group ,but no significant difference was observed between siRNA group and the controls (*P*＞0.05).