Research Article

Neuroimmunomodulation

Neuroimmunomodulation 2020;27:123–130
DOI: 10.1159/000510714

Received: June 4, 2020
Accepted: July 30, 2020
Published online: February 3, 2021

Expression and Significance of MicroRNA-126 and MicroRNA-21 in Peripheral Blood Mononuclear Cells in Patients with Myasthenia Gravis

Pan Huang^a Xiao-ying He^b Min Xu^c

^aDepartment of Neurology, People's Hospital of Deyang City, DeYang, China; ^bDepartment of Neurology, The Affiliated Hospital of Southwest Medical University, Luzhou City, China; ^cDepartment of Neurology, The Second People's Hospital of Deyang City, DeYang, China

Keywords

Myasthenia gravis · microRNA-126 · microRNA-21 · FOXP3mRNA · Interleukin 6 · Acetylcholine receptor antibody

Abstract

Objective: The aim of the study was to evaluate the expression and significance of microRNA-126, microRNA-21, FOXP3mRNA, acetylcholine receptor antibody (AChR-Ab), and interleukin 6 (IL-6) in peripheral blood of patients with myasthenia gravis (MG). **Methods:** From September 2015 to March 2018, 50 patients with MG who were first diagnosed as MG were selected as the MG group, and 50 healthy people in the same period were selected as the normal group. RT-PCR technology was used to detect the relative expression of microRNA-126, microRNA-21, and FOXP3mRNA in peripheral blood mononuclear cells of the MG group and the control group. ELISA and IPA technique was used to detect the expression levels of IL-6 and AChR-Ab in the peripheral serum of the 2 groups. **Results:** There were no differences between groups regarding patient's characteristics and baseline data. microRNA-21 and microRNA-126 are highly conserved in the human genome. microRNA-21, microR-

NA-126, FOXP3mRNA, AChR-Ab, and IL-6 are differentially expressed in the MG group and the control group ($p < 0.05$). microRNA-21 is positively correlated with AChR-Ab and IL-6 in MG patients ($r = 0.746, 0.789, p < 0.05$), but has no correlation with FOXP3mRNA ($r = -0.249, p = 0.055$), while microRNA-126 is positively correlated with FOXP3mRNA and negatively correlated with AChR-Ab and IL-6 ($r = 0.526, -0.797, -0.801, p < 0.05$). **Conclusion:** Patients with MG have differential expression of microRNA-21 and microRNA-126 and may participate in the pathogenesis of MG by regulating pathways related to inflammatory immune response.

© 2021 S. Karger AG, Basel

Introduction

Myasthenia gravis (MG) is a chronic, autoimmune neurological disorder that causes weakness in skeletal muscle, fatigues, and the symptoms aggravate after exercise and relieve after rest [1, 2]. The pathogenesis of MG is not yet fully understood, but this is an autoimmune disease that occurs at the nerve-muscle junction, causing transmission dysfunction. Humoral immunity and cellular immunity have been recognized to play an impor-

tant role in the pathogenesis of the disease [3]. Previous studies have focused mostly on downstream factors in the immune response signal pathway such as acetylcholine receptor antibody (AChR-Ab) or T-cell-related factors such as interleukin 6 (IL-6). However, very few studies include key upstream regulatory factors in related pathways, and the mechanisms of associated pathways are not very clear [4, 5]. Therefore, there is an urgent need to explore further research on the upstream and downstream key factors in the relevant immune pathways of MG for drawing a complete map of the pathogenesis of MG.

MicroRNA is a type of single-stranded non-coding small RNA with a length of approximately 17–22 nucleotides. Due to its stable expression, it can be detected in peripheral blood and has become a biological marker for many diseases [6, 7]. MicroRNA binds to the 3 noncoding regions of the target mRNA through the principle of complementary base pairing, inhibits protein translation of the related target gene, and plays a regulatory role [8, 9]. Recently, several autoimmune diseases have been identified as differential expression profiles of microRNA, suggesting that microRNA has a role in regulating the immune response [10–12]. FOXP3 is a transcription factor that controls the body's own immune tolerance response. Decreased expression of FOXP3 also suggests an improvement in the body's own immune response, while increased expression has the effect of preventing autoimmune diseases [13]. Therefore, the change of its expression may, to some extent, reflect the therapeutic effect of autoimmune diseases to a certain extent [14, 15]. Is there a differential expression profile of immune-related microRNA and FOXP3mRNA in MG patients? What role do these microRNAs and FOXP3mRNA play in the pathogenesis of MG? To this end, this study explored the expression levels and correlation of microRNA-126, microRNA-21, FOXP3mRNA, AChR-Ab, and IL-6 in the peripheral blood of patients with MG and presented new laboratory evidence to expose the immune pathogenesis of MG further.

Materials and Methods

Clinical Data

Sixty patients with MG, who were first diagnosed with MG, were admitted to our hospital from September 2015 to March 2018 and were selected as the experimental group (MG group). Among them, there were 29 males and 31 females with 28–53 years of age and an average of (33.63 ± 5.68) years. Besides, 50 cases with healthy physical status have been selected as a normal group during the same time. Among them, 24 males and 26 females had 29–56 years of age, with an average age of (34.14 ± 5.76) . There was

no statistically significant difference in terms of sex and age between the 2 groups ($p > 0.05$). This study was approved by the hospital Ethics Committee.

Inclusion Criteria

(1) Meet the Chinese expert consensus on the diagnosis and treatment of MG [16]. (2) Patients with complete clinical data of MG. (3) No cerebral infarction or cerebral hemorrhage disease confirmed by CT or MRI. (4) Those who are conscious and can carry out this research. (5) Patients or their family members signed informed consent. (6) Patients who are diagnosed with MG for the first time and have not been treated.

Exclusion Criteria

(1) Combined with severe heart, liver, kidney, or lung insufficiency. (2) Combined with severe disease. (3) Combined with autoimmune disease or using drugs that suppress immune function. (4) Combined with a malignant tumor and not first diagnosed with MG.

microRNA-126, microRNA-21, and FOXP3mRNA Detection

Separated and extracted peripheral blood mononuclear cells from patients with MG and then took a sample of 200 μ L and melted it onto the ice. The melted sample was then added into an EP tube containing 1 mL of Trizol and extracted RNA according to the Trizol method. A nucleic acid protein analyzer was used to determine the purity of RNA, and the value of O_{260}/O_{280} between the range of 1.8 and 2.0 was considered as qualified. The synthesis of first-strand cDNA synthesis was performed using the M-MLV reverse transcriptase kit (Invitrogen™). After preparing the reaction system, including internal reference gene-specific reverse transcription primer (10 μ M) 1.0 μ L, target gene-specific reverse transcription primer (10 μ M) 1.0 μ L, dNTP Mix 1.0 μ L, RNA 1.0 μ L, 5*first-strand synthesis buffer, 4.0 μ L, 0.1 M DTT 2.0 μ L, and M-MLV reverse transcriptase (1.0 μ L), reacted at 95°C for 5 min and 37°C for 50 min to perform reverse transcription and amplification into cDNA. Then, for preparing the RT-PCR reaction mixture, a 96-well reaction plate was taken, in which the mixture was made with 2xqPCR mix of 5.0 μ L, primer working solution (2.5 μ M) of 1.0 μ L, the template of 1.0 μ L, ddH₂O of 2.8 μ L, and rox of 0.2 μ L, and the total volume was 10 μ L. Each sample was made in 3 replicate wells in which the conditions of amplification were 95°C, 1 min; 95°C, 15 s, 58°C, 20 s, and 72°C, 45 s, with a total of 40 cycles. Since the Ct value in the PCR could not be used as the original data to be analyzed in statistics, it must be standardized. This study uses $2^{-\Delta Ct}$ to represent the relative expression of microRNA, $\Delta Ct = (\text{Target microRNA Ct value} - \text{U6 Ct value})$. The relevant primer sequences are discussed in the following section (Table 1).

IL-6, AChR-Ab Detection

The ELISA method was used to detect the expression level of IL-6 in the peripheral serum of the 2 participating groups. The IPA method was used to identify the positive rate and expression level of AChR-Ab in the peripheral serum of the 2 groups of participants (the AChR-Ab concentration was higher than 0.5 nmol/L is positive).

Statistical Analysis

The assumption of normality for variables was tested by the Shapiro-Wilk test. Variables with normal distribution were tested

Table 1. Primer sequence

Primer name	Primer sequence
<i>U6</i>	
RT primer	5'-AACGCTTCACGAATTTGCGT-3'
Forward	5'-CTCGCTTCGGCAGCACAT-3'
Reverse	5'-AACGCTTCACGAATTTGCGT-3'
<i>miR-21-5p</i>	
RT primer	5'-CTCAACTGGTGTCTGTTGGAGTCGGCAATTCAGTTGAGTCAACATC-3'
Forward	5'-TGGGCTTATCAGACTGATGTTGA-3'
Reverse	5'-CTCAACTGGTGTCTGTTGGAGTC-3'
<i>FOXP3mRNA</i>	
RT primer	5'-CTCAACTGGTGTCTGTTGGAGTCGGCAATTCAGTTGAGTCAACATC-3'
Forward	5'-GGGTAGCCATGGAAACAGCAAGTA-3'
Reverse	5'-TCGCATGTTGTGGAAGTTGAAGTAG-3'
<i>miR-126-3p</i>	
RT primer	5'-CTCAACTGGTGTCTGTTGGAGTCGGCAATTCAGTTGAGTCAACATC-3'
Forward	5'-TCGTACCGTGAGTAATAATGCG-3'
Reverse	5'-CTCAACTGGTGTCTGTTGGAGTC-3'

Table 2. Characteristics of the MG group and control group

Item	MG group (<i>n</i> = 60)	Control group (<i>n</i> = 50)	<i>t</i> / χ^2	<i>p</i> value
Sex, M/F	29/31	24/26	0.27	0.61
Age, years	33.6±5.6	34.14±5.76	0.46	0.64
BMI, kg/m ²	23.1±2.2	24.65±3.16	1.37	0.17
Hypertension, <i>n</i> (%)	14 (23.33)	14 (28.00)	1.48	0.21
Diabetes, <i>n</i> (%)	7 (11.67)	6 (12.00)	1.87	0.16
TG, mmol/L	1.41±0.21	1.44±0.22	0.72	0.46
TC, mmol/L	4.09±0.87	4.11±0.91	0.58	0.55
HDL-C, mmol/L	0.88±0.32	0.81±0.29	1.22	0.23
LDL-C, mmol/L	2.68±0.82	2.87±0.86	1.17	0.24

MG, myasthenia gravis; TG, triglyceride; TC, total cholesterol; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

with the Student's *t* test for baseline analyses, and the paired-samples *t* test for longitudinal analyses. Variables with nonnormal distribution were tested with the Mann-Whitney U test for baseline analyses and the Wilcoxon signed-rank test for longitudinal analyses. Effect sizes for each test were calculated accordingly. The Pearson and Spearman correlations tests were used for variables with normal and nonnormal distribution, respectively. *p* < 0.05 was considered statistically significant. SPSS software (for Windows, version 20.0, IBM, Armonk, NY, USA) was used for computing statistical tests, and graphs were generated by GraphPad Prism[®] software (v5.0, San Diego, CA, USA).

Result

All participants from MG group and Control group completed the study and were included in the analysis. The demographic characteristics, comorbid diseases, and laboratory tests are shown in Table 2. All variables were not significantly different between the two groups.

In the preliminary test, 3 patients were selected from the MG group, and 1 healthy person was selected from the normal group for the stability test of microRNA-21 and microRNA-126. The results show that both microRNAs

Fig. 1. Comparison of microRNA-21 and microRNA-126 in the peripheral blood of the MG group and control group. MG, myasthenia gravis.

start to amplify around the 14th cycle, and the amplification ends around the 30th cycle. The Ct value is meaningful, indicating that the selected 2 microRNAs meet the requirements and the experimental conditions is good.

The position and degree of conservation of microRNA-21 and microRNA-126 in the human genome can be found through the UCSC database (<http://genome.ucsc.edu>). MicroRNA-21 is located at 5981337–59841337 in the long arm 23 region 1 of chromosome 12 in the human genome, and the length is about 100 bp. Conservation analysis shows that the microRNA-21 nucleotide sequence is highly conserved among 8 species: human, rhesus monkey, mouse, dog, elephant, chicken, x-tropicalis, and zebrafish (online suppl. material 1; see www.karger.com/doi/10.1159/000510714 for all online suppl. material). MicroRNA-126 is located at 136670602–136670686 in the long arm 34 region 3 of chromosome 9 in the human genome, and the length is about 85 bp. Conservation analysis shows that the microRNA-126 nucleotide sequence is highly conserved among 7 species: human, chicken, dog, mouse, elephant, x-tropicalis, and rhesus monkey (online suppl. material 2).

Table 3 and Figure 1 show the relative expression levels of microRNA-126 and microRNA-21 in the MG group and the control group. Statistical analysis demonstrated that the relative expression levels of microRNA-21 and microRNA-126 in the peripheral blood mononuclear cells of the MG group and the control group are significantly different ($t = 21.18, 32.10$ $p < 0.05$).

Table 3. Comparison of microRNA-21 and microRNA-126 in peripheral blood of MG group and control group patients

Item	MG group (n = 60)	Control group (n = 50)	t	p value
microRNA-126	0.97±0.24	2.48±0.49	-21.18	<0.05
microRNA-21	3.50±0.52	1.01±0.19	32.10	<0.05

MG, myasthenia gravis.

Table 4. Comparison of FOXP3mRNA, AChR-Ab, and IL-6 in peripheral blood of MG group and control group patients

Item	MG group (n = 60)	Control group (n = 50)	t	p value
FOXP3mRNA	1.31±0.43	3.17±0.91	-14.06	<0.05
AChR-Ab, nmol/L	0.69±0.32	0.39±0.13	6.21	<0.05
IL-6, ng/mL	180.19±42.67	114.09±34.89	8.77	<0.05

MG, myasthenia gravis; IL-6, interleukin 6; AChR-Ab, acetylcholine receptor antibody.

Table 5. Correlation analysis of microRNA-21, microRNA-126 and FOXP3mRNA, AChR-Ab, and IL-6 in MG patients

Item	MicroRNA-21		MicroRNA-126	
	r	p value	r	p value
FOXP3mRNA	-0.249	0.055	0.526	<0.05
AChR-Ab	0.746	<0.05	-0.797	<0.05
IL-6	0.789	<0.05	-0.801	<0.05

MG, myasthenia gravis; IL-6, interleukin 6; AChR-Ab, acetylcholine receptor antibody.

Table 4 shows the levels of FOXP3mRNA, AChR-Ab, and IL-6 in peripheral blood of the MG group and the control group. The positive rate of AChR-Ab in the peripheral serum of the MG group was 45.00% (25/60 cases), and the AChR-Ab level was (0.69 ± 0.32) nmol/L, which was significantly higher than that of the control group (0.39 ± 0.13) nmol/L. p values < 0.05 were considered statistically significant. The levels of FOXP3mRNA were 1.31 ± 0.43 and 3.17 ± 0.91 in the MG and Control groups at baseline, respectively ($p < 0.05$). In addition the IL-6 levels in the MG group and the control group were 180.19 ± 42.67 and 114.09 ± 34.89 ng/mL, respectively ($p < 0.05$).

Fig. 2. a-f Correlation analysis of microRNA-21, microRNA-126 and FOXP3mRNA, AChR-Ab, and IL-6 in MG patients. MG, myasthenia gravis; IL-6, interleukin 6; AChR-Ab, acetylcholine receptor antibody.

Table 5 and Figure 2a–f show that microRNA-21 is positively correlated with AChR-Ab and IL-6 in MG patients ($r = 0.746, 0.789, p < 0.05$), but has no correlation with FOXP3 mRNA ($r = -0.249, p = 0.055$), while microRNA-126 is positively correlated with FOXP3 mRNA and negatively correlated with AChR-Ab and IL-6 ($r = 0.526, -0.797, -0.801, p < 0.05$).

Discussion

MG often has a sudden onset and recurrent attacks. In severe cases, respiratory failure may occur due to the involvement of the respiratory muscles. Such patients have a poor prognosis. At present, the pathogenesis of MG has not been fully elucidated. Most researchers believe that it is an autoimmune disease in which the AChR-Ab plays a vital role in the pathogenesis of MG. In the present study, the expression level of AChR-Ab in the peripheral blood of patients with MG increased, suggesting that AChR-Ab is closely related to MG pathogenesis. Studies have shown that patients with MG have an expression of AChR-Ab with more than 85% of the whole-body, while more than 50% of ocular muscle type [17, 18]. Acetylcholine (ACh) is a common neurotransmitter for excitation. ACh binds to ACh receptors (AChR) to cause muscle contraction when the excitement reaches the efferent nerve end. Among MG patients, a significant amount of AChR-Ab is developed in the body, which can compete with the combination of ACh and AChR to form specific complexes, leading to accelerated degradation of AChP, and ultimately resulting in neuromuscular transmission dysfunction due to the reduction of ACh on the postsynaptic model [19]. Furthermore, this study observed an increase in IL-6 expression in MG patients. T lymphocyte subsets can be divided into many types by function and surface markers. (1) Helper T cell (Th) can activate other types of immune cells through proliferation and diffusion, and its primary surface marker is CD4. (2) Cytotoxic T cell can kill target cells providing an appropriate response to antigen, and its primary surface marker is CD8. (3) Regulation/suppressor T cell (Treg) is responsible for controlling the immune response of the body. It has an inhibitory effect on various T cells and B cells, maintains and controls the immune response, and maintains immune homeostasis (immune tolerance). Among these, CD4 T cells may differentiate into Th2 cells, which mainly secrete IL-4, IL-6, and other cytokines, thus promoting proliferation and differentiation of B cells and the formation of antibodies. Therefore, Th2

cells play a primary role in assisting specific B lymphocytes proliferate and produce AChR-Ab by initiating a humoral immune response [20].

This study observed a decrease in the relative expression of FOXP3 mRNA in peripheral blood mononuclear cells in patients with MG. Forkhead/winged-helix transcription factor 3 (Foxp3) is a specific transcription factor that has been discovered in the present studies. It is widely expressed in vivo and mediates CD4⁺ CD25⁺ Treg cells as a role of expression and maintenance of function in the periphery [21–23]. When FOXP3 is inactivated or mutated, this may lead to a decrease in the activity of NF- κ B, resulting in the loss of regulatory T cells and fatal self-aggression lymphocyte proliferation [24, 25]. Hence, some people compare it to a rheostat of the immune response. The primary function of CD4⁺ CD25⁺ Treg is to inhibit the activation and proliferation of CD4⁺ CD25⁺ T cells in order to achieve a negative regulation of immune responses. Human autoimmune disorders are often characterized by a relative lack of cells with CD4⁺ CD25⁺ Treg. Based on this principle, Liu [26] injected an immune complex of IL-2 and anti-IL-2 mAb into the experimental autoimmune myasthenia gravis (EAMG) model and found that Treg cells grew continuously and effectively in the experimental model. Proliferating Treg cells may inhibit the self-reaction of T cells and B cells to AChR while alleviating symptoms of muscle weakness [27]. At the same time, researchers have found that the decrease in the ratio of Treg cells is closely related to the EAMG pathogenesis of EAMG, thus regulating the level of Treg cells for the future treatment of MG [28].

MicroRNA is a type of small non-coding RNA with a length of approximately 17–22 nucleotides [29]. It is combined with the principle of complete or incomplete base complementary pairing with the target gene mRNA to play a role in the transcriptional expression of the target gene [30]. The purpose of negative regulation is much involved in regulating the growth and development of the body and the occurrence of diseases. MicroRNA-126 has several effects. Researchers have found that the up-regulated microRNA-126 can inhibit the ability of the SKOV3 ovarian cancer cell to migrate and invade in vitro, suggesting that the low-expressed microRNA-126 may be associated with the progression of ovarian cancer [31]. Another group of researchers has found that microRNA-126 can participate in the regulation of angiogenesis by regulating multiple target angiogenesis-related genes, such as VEGF, VCAM-1, and PAK1 [32, 33]. The latest research shows that microRNA-126 also has a role in regulating

the immune system. For example, the area of ulcer and the infiltration of inflammatory cells in ulcerative colitis model rats that knockdown microRNA-126 are heavier than non-microRNA-126 knockdown rats [34]. For children with bronchial asthma, the expression level of microRNA-126 is significantly increased [35]. Besides, Qin found that microRNA-126 has a role in controlling peripheral induction and immunosuppression of CD4+FOXP3+ T cells [36]. For microRNA-21, the up-regulation of microRNA-21 expression was found in the thymus tissues of patients with MG combined with thymic tumors. However, microRNA-126 and microRNA-21 have immunoregulatory effects that are mostly focused on cell or animal (mice) research, and clinical research is lacking. MG is an immunoreactive disorder, which is well known [37]. Hence, the present research used RT-PCR to detect the expression of microRNA-21 and microRNA-126 in the peripheral blood of patients with MG and performed a preliminary analysis of its possible mechanism.

Results obtained from the present studies have confirmed that the structure of a substance determines its function, and substances with similar molecular structures may perform the same functions. Since microRNA may have differences in the conservation of nucleotide sequences between different species. Thus, microRNA-126 and microRNA-21 have a role in regulating immunity in cell experiments or experiments with animals. However, in humans, it may function differently because of the nucleotide sequence [38]. In the present research, we first analyzed the high structural homology of microRNA-126 and microRNA-21 between human, mice, and other species through the UCSC database, suggesting that both microRNAs may also play a role in regulating themselves in humans. Furthermore, microRNA-21 was found to be high, while microRNA-126 was low in patients with MG, confirming our assumption that both microRNAs are related to MG pathogenesis. Nevertheless, further correlation analysis showed that microRNA-21 has no apparent association with FOXP3mRNA, and is positively correlated with AChR-Ab and IL-6, while microRNA-126 is positively correlated with FOXP3mRNA and negatively correlated with AChR-Ab and IL-6. It is suggested that the expression of microRNA-21 and microRNA-126 can be regulated by FOXP3mRNA and IL-6. When the expression of FOXP3mRNA and IL-6 changes, it may influence the expression of AChR-Ab to mediate MG pathogenesis through the mechanism discussed earlier in this article. However, due to time and experimental limitations, only the correlation analysis between mi-

croRNA-21 and microRNA-126 has been presented here, but could not carry out the immune response-related indicators based on the molecular pathological level of their specific regulatory mechanisms. Furthermore, the sample size of this study is small, and this single-center study may lead to a certain degree of bias in the results. More large-scale multicenter studies are required for verification in the future.

Acknowledgement

The authors would like to thank the patients who helped us to realize this project.

Statement of Ethics

The study was approved by the Institutional Ethics Committee of People's Hospital of Deyang City (ethical approval number: 2015-03-002). All patients provided written informed consent.

Conflict of Interest Statement

The authors declare that they have no conflict of interest to declare.

Funding Sources

There are no funding sources.

Author Contributions

Pan Huang contributed to the conception of the study. Pan Huang and Min Xu contributed significantly to analysis and manuscript preparation; Pan Huang and Min Xu performed the data analyses and wrote the manuscript; Xiao-ying He helped perform the analysis with constructive discussions.

References

- 1 Gilhus NE, Verschuuren JJ. Myasthenia gravis: subgroup classification and therapeutic strategies. *Lancet Neurol*. 2015;14(10):1023–36.
- 2 Carr AS, Cardwell CR, McCarron PO, McConville J. A systematic review of population based epidemiological studies in myasthenia gravis. *BMC Neurol*. 2010;10:46.
- 3 Chinese Society of Immunology Neuroimmunology Branch. Chinese medical association neurology branch god the immunology group. Chinese expert consensus on diagnosis and treatment of myasthenia gravis. *Chin J Neuroimmunol Neurol*. 2012;19(6):401–8.

- 4 Dalakas MC. Novel future therapeutic options in myasthenia gravis. *Autoimmun Rev.* 2013;12(9):936–41.
- 5 Zhang YY, Cao JB, Zhang H. Role of microRNA in the regulation of cognitive function: a potential as novel biomarker. *Acad J Chin PLAM Sch.* 2017;38(2):185–9.
- 6 Wang XX, Sun LH. Progress in regulation of interactions between long non-coding RNA and microRNA. *Chin J Clin Pharmacol.* 2018; 34(05):589–92.
- 7 Urbich C, Kuehnbacher A, Dimmeler S. Role of microRNAs in vascular diseases, inflammation, and angiogenesis. *Cardiovasc Res.* 2008; 79(4):581–8.
- 8 Xiao Y, Wang H, Wang C, Zeng B, Tang X, Zhang Y, et al. miR-203 promotes HaCaT cell overproliferation through targeting LXR- α and PPAR- γ . *Cell Cycle.* 2020(4):1–13.
- 9 Lu H, Yang F, Liu B, Zhang K, Cao Y, Dai W, et al. Intracellular low-abundance microRNA imaging by a NIR-assisted entropy-driven DNA system. *Nanoscale Horizons.* 2019;4(2): 472–9.
- 10 Pang CY, Wang YF. Role of microRNA on diagnosis and treatment in rheumatoid arthritis. *J Med Postgra.* 2014;27(5):554–6.
- 11 Liu W, Zheng J, Dong J, Bai R, Song D, Ma X, et al. Association of miR-197-5p, a circulating biomarker for heart failure, with myocardial fibrosis and adverse cardiovascular events among patients with stage C or D heart failure. *Cardiology.* 2018;141:212–25.
- 12 Yurt M, Ayyildiz O, Karakus A, Nursal AF, Isi H. MicroRNAs expression profiles as biomarkers and therapeutic tools in Turkish patients with chronic myeloid leukemia. *Bratisl Lek Listy.* 2020;121:159–63.
- 13 Xiong J, Tan R, Wang W, Wang H, Pu D, Wu J. Evaluation of CD4+CD25+FOXP3+ regulatory T cells and FOXP3 mRNA in premature ovarian insufficiency. *Climacteric.* 2020; 23(3):267–72.
- 14 Liu Y, Wang L, Han R, Beier UH, Hancock WW. Two lysines in the forkhead domain of foxp3 are key to T regulatory cell function. *PLoS One.* 2012;7(1):e29035.
- 15 Wang L, Jin JY, Chen LH. Immune response involved in the regulation of CD4+ T cells. *Immunol J.* 2018;34(01):73–
- 16 Chinese expert consensus on diagnosis and treatment of myasthenia gravis. *Chin J Neuroimmunol Neurol.* 2012;19(06):401–8.
- 17 Chu F, Hu Y, Guo M, Chao C, Zhao J, Yun L, et al. Role of microRNA-126 in CD4+ T cell mediated autoimmune colitis in mice. *J Third Mil Med Univ.* 2017;39(08):767–72.
- 18 Xu CD, Tang X, Zhao DY, DO Respiration. Expression differences of microRNA-126 in mast cell and its influence on release of IL-4. *Chin J Prac Pediatr.* 2017;32(12):947–50.
- 19 Qin AD, Liu J, Luo JM, Xu L. MicroRNA-126 regulates the peripheral induction and function of CD4+Foxp3+ T cells. *J Immunol.* 2016;32(05):382–7.
- 20 Tzartos JS. Autoantibodies to lipoprotein-related protein 4 in patients with double-seronegative myasthenia gravis. *Arch Neurol.* 2012;69(4):445–51.
- 21 Marangoni F, Zhang R, Mani V, Thelen M, Ali Akbar NJ, Warner RD, et al. Tumor tolerance-promoting function of regulatory T cells is optimized by CD28, but strictly dependent on calcineurin. *J Immunol.* 2018;200(4): 3647–61.
- 22 Wu Y, Borde M, Heissmeyer V, Fehlings M, Lapan AD, Stroud JC, et al. FOXP3 controls regulatory T cell function through cooperation with NFAT. *Cell.* 2006;126(2):375–8.
- 23 Vaeth M, Müller G, Schmitt D, Dietz L, Klein Hessling S, Serfling E, et al. Intracellular regulatory T cells control humoral immunity via NFAT2-regulated CXCR4 expression. *J Exp Med.* 2014;211(1):55–61.
- 24 Chen S, Fang L, Guo M, Hu G, Li W, et al. Control of cell cycle arrest and immune equilibrium by Lkb1 in dendritic cells. *Nat Commun.* 2018;9(1):5298.
- 25 Yang K, Boudreau N, Gaudet G, Vogel P, Avila J, Werb CB, et al. The costimulatory control of metastasis and functional fitness of Treg cells by Lkb1 signaling. *Nature.* 2017;548(7669): 602–6.
- 26 Liu R, Zhou Q, La Cava A, Campagnolo DI, Vignola L, Shi FD. Expansion of regulatory T cells via IL-2/anti-IL-2 mAb complexes suppresses experimental myasthenia. *Eur J Immunol.* 2010;40(6):1577–89.
- 27 Du WW, Li JP, Chen ZZ, Liu JL, Mo XA, Sun SG. The effect of autoimmune regulatory factors on the pathogenesis of EAMG model mice. *Chin J Neuroimmunol Neuro.* 2014; 21(3):157–60.
- 28 Truffault F, Nazzari Di, Verdier J, Gradolatto A, Fadel E, Roussin R, et al. Comparative analysis of thymic and blood treg in myasthenia gravis: thymic epithelial cells contribute to thymic immunoregulatory defects. *Front Immunol.* 2020;11:782.
- 29 Jin JC. The overview and research of microRNA. *J Med Postgra.* 2013;26(10):1109–1.
- 30 Wienholds E, Kloosterman AD, Miska E, Alvarez-Saavedra E, Berezikov E, Brummelman J, et al. MicroRNA expression in zebrafish embryo development. *Science.* 2005;309(5732): 310–1.
- 31 Luo P, Fei L, Zhou JW, Wang W. miR-126 suppresses PAECs expression in ovarian cancer SKOV3 cells. *Cell Prolif.* 2015; 9(5):2225–9.
- 32 Lou Z, Guo M, Wang L, Li Y. The effects of microRNA-126 reduce inflammation and apoptosis in diabetic nephropathy through PI3K/AKT signaling pathway by VEGF. *Cell Physiol Biochem.* 2020;45(1):1–10.
- 33 Xu XS, Chen JJ, Dai M. Paeonol promotes microRNA-126 expression to inhibit monocyte adhesion to ox-LDL-injured vascular endothelial cells and block the activation of the PI3K/Akt/NF-KB pathway. *Int J Mol Med.* 2016;38(6):1871–8.
- 34 Chu F, Hu Y, Guo M, Chen C, Zhao J, Liu Y, et al. Role of microRNA-126 in CD4+ T cell-mediated autoimmune colitis in mice. *J Third Mil Med Univ.* 2017;39(08):767–72.
- 35 Xu CD, Tang X, Zhao DY, Liu F. Expression differences of microRNA-126 in mast cell and its influence on release of IL-4. *Chin J Prac Pediatr.* 2017;32(12):947–50.
- 36 Chu F, Hu Y, Zhou Y, Guo M, Lu J, Zheng W, et al. MicroRNA-126 deficiency enhanced the activation and function of CD4+ T cells by elevating IRS-1 pathway. *Clin Exp Immunol.* 2018;191(2):166–79.
- 37 Qiu D, Li J, Xue AM, Chen Z. Preliminary study of microRNA expression profiles in the abnormal thymus of generalized myasthenia gravis. *Chin J Neuroimmunol Neuro.* 2015; 22(3):171–81.
- 38 Sheng XH, Du LX. MicroRNA and its research progress in humans and animals. *Genetics.* 2007(06):651–8.