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Influence and significance of intervening diabetes microRNA expression profile of NOD mice with exendin-4

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Abstract. – OBJECTIVE: To provide selectable microRNA for intervening diabetes mellitus diseases, NOD mice's expression of microRNA in pancreas tissues and blood under the exendin-4 intervention of was observed and the difference of microRNA target gene was screened.

MATERIALS AND METHODS: Forty clean NOD mice were randomly divided into four groups (in each group, n = 10): One is blank control group D which is intervened with normal saline, and the other three groups were divided into low-dose group A, middle-dose group B, and high-dose group C according to the different exendin-4 dosage 2, 4, and 8 μg/kg·d. After the 8-week intervention, these four groups were killed, and the pancreatic tissue and blood were left to prepare specimens for morphology and molecular biology analysis. The specimen with differential expression microRNA in pancreas tissue and blood should be screened out after detected with the locked nucleic acid array system (LNATM) microRNA expression profile chip. The primers should be designed, and the ABI7500 real-time fluorescent quantitative PCR should be applied to amplify, analyze, and verify according to the screen results of the microRNA chip in order to screen out the significant differentially expressed microRNA.

RESULTS: Histological detection showed that the pancreas of the mice in control group D was fibrosis gradually and the islet frame was relatively disordered and significantly atrophied. Groups A, B, and C have no islet hypertrophy or atrophy and the degree of fibrosis of the pancreas has reduced. According to the gene chip detection, there are four significantly differently expressed microRNAs in pancreas tissue and blood among the group A, B, and C, among which miR-19a, miR-19b, and miR-22 were downregulated expressed while the miRNA-1 was upregulated expressed. Bioinformatics analysis showed that the target genes of 4 differentially regulated microRNA genes were related to cell proliferation, apoptosis, glucose metabolism, and angiogenesis. The expression of microRNA in pancreatic tissue and blood of NOD rats was highly consistent.

CONCLUSIONS: MicroRNA expression file of pancreatic tissue and blood can be changed during the intervention of the NOD rat model with exendin-4. MicroRNA that indicates the differential expression may take part in the recovering process of the NOD pancreatic trauma. At the same time, the administration of exendin-4 can protect NOD mice, reduce its pancreatic tissue fibrosis, and regulate molecular markers of pancreatic cells in size and pancreatic mast cells. This may be one of the main mechanisms of pancreatic injury in diabetes prevention.

Key Words:
Exendin-4, microRNA, Non obese, Diabetes mellitus, Pancreatic injury.

Introduction

Diabetes is a kind of serious non-communicable disease, and recently years has witnessed an increasing number of cases of this disease. Clinically, patients with NOD make up the majority of diabetes patients due to inadequate insulin resistance and insulin[1]. Researches show[2-4] that microRNA (miRNA) plays an important role in some diseases, such as coronary heart disease and gastric cancer, while few reports mentioned its role in the NOD. Exendin-4, polypeptide consisted of 39 amino acids, is a kind of new drug to treat diabetes, which can lower the blood glucose level both when patients are in hungry or full and have a relatively high tolerance to dipeptidyl peptidase-IV[5]. This study aims at discussing the microDNA expression in NOD rat pancreas under the exendin-4 intervention and the effect of mi-

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croRNA in NOD pancreas trauma by screening the differential microRNA target gene.

Materials and Methods

Main Reagents and Instruments

Exendin-4 reagents (Kang Tai Biotechnology (Beijing) Co., Ltd., Beijing, China), total RNA extraction reagent TRIZOL (Shang-hai Ying Gong Reagent Co., Ltd., Shanghai, China), microRNA isolation kit (Hangzhou Woosen Biotechnology Co., Ltd., Hangzhou, China), HE dyeing (Beijing Solarbio Technology Co., Ltd., Beijing, China), LNATM microRNA chip (Exiqon Company version 16.0, Vedbaek, Denmark), blood glucose meter (ONETOUCH UltraVue), ABI7500 real-time fluorescence quantitative PCR instrument (Shanghai Ke Hua Experimental Systems Co., Ltd., Shanghai, China), optical microscope (Shenzhen Finial Technology Co., Ltd., Shenzhen, China), 2100 biological analyzer (Agilent Technology Co., Ltd., Santa Clara, CA, USA).

Grouping and Modeling

Forty NOD rats at six weeks old, half males and half females, which were provided by a Medical University Animal Experimental Center, were used to establish the diabetic rat models. Blood sugar was measured in all NOD rats with blood glucose meter 48 h after model establishment. If the blood glucose level is more than 16.7 mmol/L, and symptoms such as polyuria, polydipsia, polyphagia, weight loss, etc. appeared, it is treated as successful diabetes models.

The establishment of exendin-4 intervention NOD rat model: forty NOD rats were divided into 4 groups (n = 10) randomly by using the method of random digits table: blank control group D with physiological saline intervention, and the other 3 groups implemented exendin-4 intervention. According to different doses of 2, 4, and 8 ug/kg·d of exendin-4 intervention, respectively, referred to as low-dose group A, middle-dose group B, and high-dose group C. Four groups of rats were intervened for 8 weeks and killed after 8 weeks, leaving the pancreas tissue and blood stored at the temperature of −80°C.

Experimental Methods

Histological Examination of the Pancreas

Taking the pancreas tissue specimens from each group and fixed them with neutral formalin fixative (10%). After the implementation of dehydration, washing and paraffin-embedding and other operations, slicing and HE staining, we began to observe the pancreatic cell morphological changes by optical microscopy.

Total RNA Extraction and Identification

Extraction of total RNA from pancreatic tissue and blood sample was carried out with specific steps of the TRIZOL reagent specification. miRNA was got after the purification of miRNeasy Mini Kit, hereafter, the absorbance values of total A260 and A280 were measured by NanoDrop 2000(Beijing Koled Traffic Co., Ltd., Beijing, China) to detect the purity of total RNA. Meanwhile, the quality was identified by using 2100 biological analyzer(Agilent Technology Co., Ltd., Santa Clara, CA, USA).

Detection of miRNA Expression

ABI7500 real-time fluorescence quantitative PCR instrument (Life Tech applied Biosystems, New York, NY, USA) was used to detect the specific miRNA and analyze its expression profile. The samples were repeated 3 times in each group. Firstly, RT^2 Easy First Strand Kit was used to synthesize cDNA. (The operation steps were carried out in accordance with kit instructions). Secondly, adding 100 µl cDNA template and 2X RT^2 SYBR Green qPCR Master Mix 125 µl, ddH_2O into 2550 µl. The mixed liquor of the same volume was distributed to the 96 hole plate, the total reaction system is 25 µl. Finally, ABI7500 real-time fluorescence quantitative PCR was used to detect, the reaction procedures were as follows: 95°C pre denaturation 10 min; 95°C denaturation 15 s; 60°C annealing 1 min; 72°C extension 30 s, amplifying 40 cycles.

Analysis of miRNA differential expression: Using ABI PRISM 7700 Sequence Detection System (Applied Biosystems, Foster City, CA, USA) exported original data to upload to the online analysis software provided by Qiagen Company (Berlin, Germany) for data analysis, so as to screen out miRNA of differential expression.

Differential miRNA Target Gene Prediction and Functional Analysis

Adopting the bioinformatics analysis method, TargetScan (http://www.targetscan.org/) and miR and a databases (http://www.microrna.org/microrna/home.do) were used to predict and made preliminary analysis of the differential expression of target gene miRNAs. Meanwhile, its function was analyzed.

Table I. Comparison of the blood glucose's general condition among the four groups.

Group	Blood glucose (mmol/L)	Weight g
A	19.66 ± 2.44*	21.49 ± 0.54*
B	18.74 ± 3.74*	22.72 ± 0.72*
C	17.24 ± 4.05*	23.04 ± 0.81*
D	23.12 ± 0.52	19.34 ± 0.22

Note: Compared with control group, $^{*}p < 0,05$

Statistical Analysis

The GraphPad 5.0 software (San Diego, CA, USA) is employed to conduct statistical processing. Intergroup comparison is expressed by t-test, using the software provided by company to express specific miRNA. $p < 0.05$ indicates that the difference is statistically significant.

Results

Comparison of the Blood Glucose's General Condition Among the Four Groups

Eight weeks after the diabetes interference model was built, blood glucose level and weight of A, B, and C group were different from control group, and the difference was statistically significant ($p < 0.05$) (Table I); diabetic symptoms such as polydipsia, polyphagia, and polyuria of rat in A, B, and C groups had relieved while that of D rat in control group was still obvious; there were no deaths among rats in four groups. The results showed that exendin-4 interference model to NOD rats was successfully built.

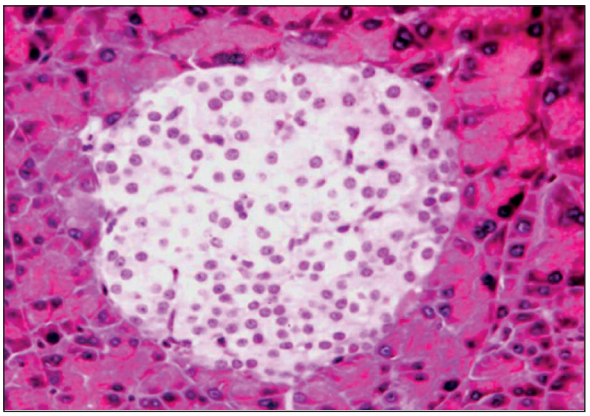

Figure 1. Morphological changes of pancreatic pathology in group A (HE, ×400).

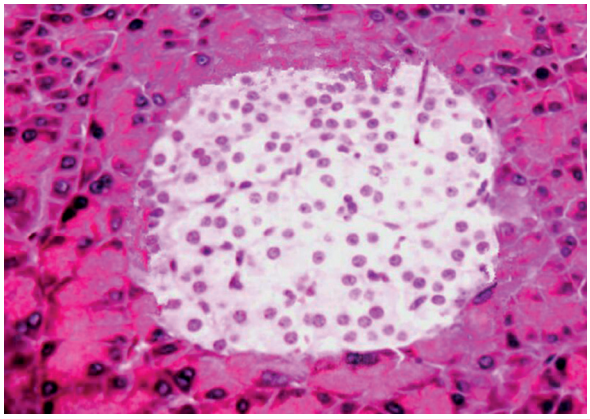

Figure 2. Morphological changes of pancreatic pathology in group B (HE, ×400).

Histological Change of the four Groups' Blood Glucose

From histological observation, pancreas islet of A, B and C treatment group did not shrink and was detected with no significant hypertrophy, and the degree of pancreatic fibrosis lessened, see Figures 1, 2, and 3; pancreas fibrosis of D rat in control group was gradually conspicuous. The islet frame was quite disorganized and was detected with significant atrophy (Figure 4).

Quality test of total RNA

By measurement, the value of A260/A 280 ranged (1.9 ± 0.1), which proves a high nucleic acid purity; total RNA integrity test finds that total RNA/miRNA have no degradation, which proves a reliable quality (Figure 5). Note that total RNA/miRNA of pancreatic tissue samples in A, B, C, and D group is in good quality and, thus, can meet the requirements of following miRNA chip inspection and PCR tests.

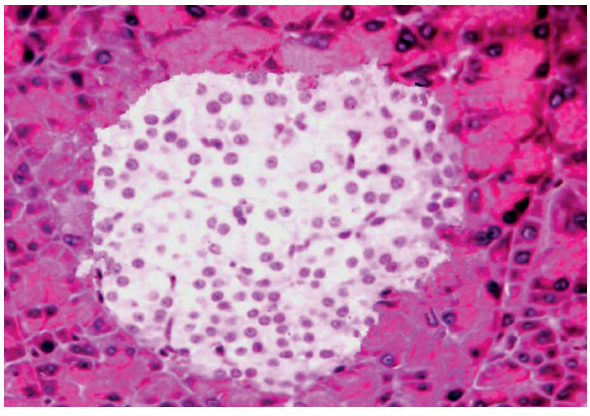

Figure 3. Morphological changes of pancreatic pathology in group C (HE, ×400).

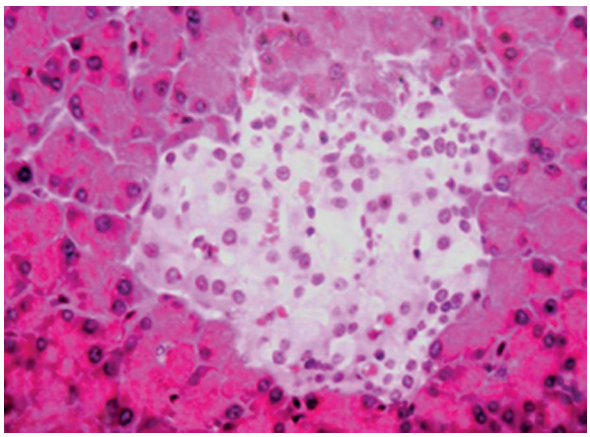

Figure 4. Morphological changes of pancreatic pathology in group D (HE, ×400).

Differential Expressed miRNA of Pancreatic Tissue and blood and its Target Prediction

Gene chip detection finds that compared with control group D, there are 4 significant differentially expressed microRNA in pancreatic tissue of A, B, and C groups, of which miR-19a, miR-19b, and miR-22 have downregulated expression and miRNA-1 has upregulated expression. By applying bioinformatics analysis softwares such as TargetScan, miRanda, PicTar, etc., we find that target gene regulated by 4 different microRNA is relevant to biological functions, such as cell proliferation, cell apoptosis, glucose metabolism and angiogenesis, see Table II.

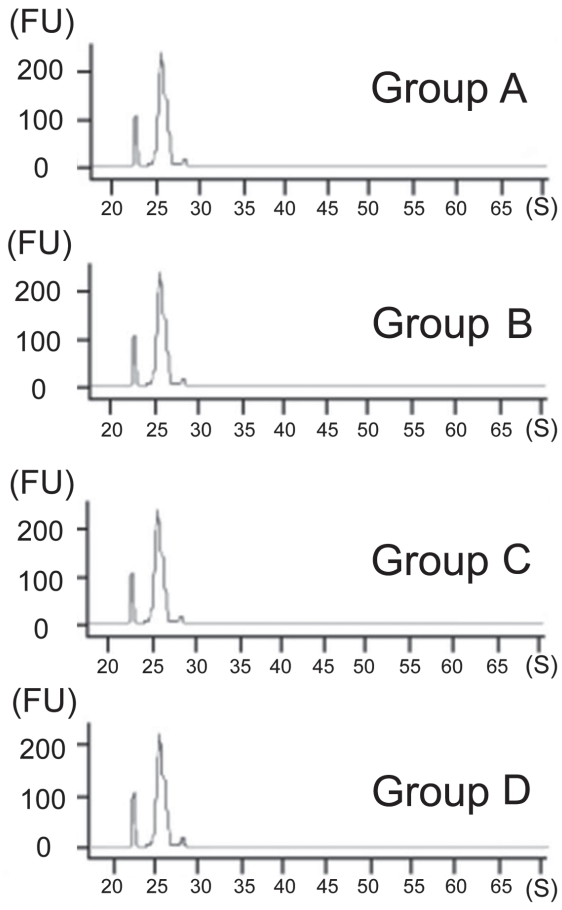

Figure 5. Integrity test of total RNA and total miRNA.

Discussion

Normal islet frame and organizational form are the basis of ensuring a normal operation of physiologic function of body islet. In various stages of nonobese diabetic development, islet organizational form generally changes, which is the basis of islet physiological functional disorders. Researches[6-9] show that the presentation of fibrosis in pancreas is closely related to cytokine secreted

Table II. Main target gene of differential expression of miRNAs

Expression change	miRNA name	Predictedtarget Gene
	miR-19a	tumor necrosis factor - α(TNF-α) gap junction protein (GJA1) cyclin D1 (CCND1) peroxisome proliferators- activated receptorsα
Downregulation	miR-19b	(N 1C1) pyruvate dehydrogenase kinase4 (PDK4)
	miR-22	serum response factor (SRF) vascular cell adhesion molecule-1 (VCAM1)
Upregulation	miR-1	insulin-like growth factor 1(IGF1)\gap junction protein (GJA1) thrombospondin- 1(TSP1)

by pancreatic stellate cell near the acinus. There are reports[10,11] that the presentation of fibrosis in pancreas islet is the final result of insulitis which has proved to be necessary stage of pathogenic process of type 1 diabetes.

Exendin-4 is a kind of polypeptide isolated from saliva of Heloderma suspectum, and shares a high similarity with glucagon-like peptide-1 (GLP-1) in structure and biological effect. It has a role on regulating blood glucose, that is when blood glucose levels become too high, it will stimulate insulin secretion in engine body, and when blood glucose is at lower concentrations or at normal level, it will not stimulate insulin secretion[12-14]. Exendin-4 and GLP-1 are hot spots of clinic treatment of diabetes. There are research reports which show that GLP-1 is difficult to be applied to clinic due to its several characters: unstable, easy to degrade when entering into human body and difficult to play its role of regulating bloodglucose. Exendin-4, also known as the receptor agonist of GLP-1, can simulate hormonal readiness of insulin in regulation body after parenteral administration, and thus can play its role in body to effectively control blood glucose.

From histological observation, the study finds that pancreas fibrosis of D rat in control group was gradually conspicuous, islet frame was significantly disorganized and was detected significant atrophy. Pancreas islet of A, B, and C treatment groups did not shrink and was detected with no significant hypertrophy, and the degree of pancreatic fibrosis lessened. It can be seen that exendin-4 can keep normal physical structure of body in some ways, reduce pathological damage of islet and to some extent reshape islet form.

miRNA is an important post-transcriptional regulatory factors, and actively participate in multiple biological process such as cell proliferation, cell apoptosis, differentiation and metabolism[15-19]. However, miRNA's role in different cell is varied. Further analysis of detected miRNA shows that compared with control group D, there are 4 significant differentially expressed microRNA in pancreatic tissue and blood of A, B, and C groups, of which miR-19a, miR-19b, and miR-22 have downregulated expression and miRNA-1 has upregulated expression. By applying bioinformatics analysis software, we find that target gene regulated by 4 different microRNA is relevant to biological functions. It indicates that in rat pancreas of exendin-4 interference nonobese diabetic, the expression of microRNA has four abnormal types, of which miR-19a, miR-19b, and miR-22 are low expression and miRNA-1 is high expression, and the expression of microRNA in pancreatic tissue and blood of NOD rats was highly consistent, all of which are speculated to show some inhibitory effect to the differentiation and maturation of pancreatic β cell, promote gradual recovery of insulin secretion function and exert protecting effect on diabetes pancreatic injury[20-22], with possibility to become the promising target of diabetes care.

Conclusions

In rat pancreas of exendin-4 interference nonobese diabetic, the expression of microRNA has changed, note that miR-19a, miR-19b, miR-22, miRNA-1, and other microRNAs of differential expression may participate in the repair process of nonobese diabetic pancreas injury. Exendin-4 interference can protect NOD rat and reduce pancreatic tissue fibrosis, regulate the pancreatic cell size and molecular marker of pancreas mast cell, which constitutes one of the main mechanism of diabetes pancreas injury prevention.

Funding

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Conflicts of interest

The authors declare no conflicts of interest.

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