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The abnormal expression level of microRNA in epithelial-mesenchymal transition of peritoneal mesothelial cells induced by high glucose

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Abstract. – OBJECTIVE: To determine the expression level of the microRNA in the process of epithelial-mesenchymal transition (EMT) of the peritoneal mesothelial cells (PMCs) induced by high glucose.

MATERIALS AND METHODS: The PMCs were cultured using M199 medium with 10% fetal bovine serum, and the EMT was induced by D-glucose stimulation. Epithelial-mesenchymal transition was determined by changes in cell morphology and the expression levels of the EMT marker genes. Changes in cell morphology were observed by inverted microscope, and the expression levels of the EMT marker genes were determined by real-time PCR. The expression levels of the microRNA were detected by real-time PCR with microRNA-specific stem-loop structure primer.

RESULTS: The PMCs changed to fusiformis following a high-glucose medium stimulated for 48 hours, and the EMT marker genes changed significantly, such as the decrease of E-cadherin and an increase of Vimentin ($p < 0.01$). These results proved the EMT had been induced by high-glucose. Applying real-time PCR with microRNA-specific stem-loop structure primer, miR-193a increased notably ($p < 0.01$), and miR-15a and let-7e decreased ($p < 0.01$), while miR-16 and miR-21 had no significant changes ($p > 0.05$). Most importantly, the increase of miR-193a was correlated with stimulus duration.

CONCLUSIONS: MicroRNA with abnormal expression levels have a primary role in regulating the EMT of PMCs induced by high glucose.

Key Words:
MicroRNA, Epithelial-mesenchymal transition, Peritoneal fibrosis, Abnormal expression.

Introduction

Continuous improvement of peritoneal dialysis, progressive peritoneal fibrosis, and high permeability of membrane and ultrafiltration failure[1,2] have been observed in some long-term dialysis patients. Although the mechanism is not clear, it may be related to long-term exposure to the high glucose, hypertonic, acid dialysate, glucose degradation products and glycosylated end products[3,4]. Epithelial-Mesenchymal Transition (EMT) is the transition process in which the epithelial cells with polarity transform into mesenchymal cells which can move freely in the extracellular matrix. Epithelial-Mesenchymal Transition promotes tissue repair and is related to chronic inflammation, cancer metastasis and development, organ fibrosis and other pathological states[5,6]. The EMT of peritoneal mesothelial cells (PMCs) is regarded as the key part in the initiation and reversible process of peritoneal fibrosis[1].

A microRNA is a single chain non-coding RNA, and is conserved in evolution with 18-25 nucleotides and inhibits the expression of target genes. It binds to the untranslated region of the target genes according to the principle of complementary base pairing[7,8]. At present, numerous microRNAs are cloned or predicted by bioinformatics methods in plant, nematode, drosophila and mammalian tissues and cells[9]. It is supposed that every microRNA can regulate hundreds of genes and one third of the genes in the human genome are regulated by microRNAs. Functional studies of microRNA find that they are involved in a variety of physiological and pathological processes such as cell proliferation, differentiation, apoptosis and cancer. In this study, EMT was induced in PMCs by high glucose and real-time PCR was used to assess expression levels of several important microRNAs.

Materials and Methods

Human PMCs were frozen cells from our department. The TRIzol used in this experiment was pur-

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chased from Invitrogen and the reverse transcriptase and SYBR Green real-time PCR kit were purchased from TAKARA. The PMCs were cultured and digested when the cells grew to 80% fusion.

After counting with a microscope, we adjusted the cell concentration with M199 medium and inoculated the whole in a 12-well culture plate (5×10^5/well). After cultivation for 24 hours in an incubator with 37 and 5% CO_2, we induced for different time with a high glucose solution (M199 medium with 50 mmol/L glucose). The M199 medium with 5.6 mmol/L glucose was used as control[10].

Total cellular RNA was extracted using the TRIzol reagent and spectrophotometer was used to determine the concentration and purity of RNA. Then, we queried the sequence of the mRNA of the target genes with a gene database (http://www.ncbi.nlm.nih.gov/). The sequences of primer for real-time PCR were designed with Primer 3 (http://frodo.wi.mit.edu/) according to the primer parameters. Reverse transcription primers and PCR primers were designed utilizing microRNA-specific stem-loop structure and synthesized by the Shanghai Invitrogen Company.

One-step SYBR® Green RT PCR kit was used to detect target genes and microRNAs. The reactions occurred at 95 for 5 s and then at 60 for 30 s in a cycle. There were 40 cycles in total. All the experiments were repeated for three times.

Statistical Analysis

The data were expressed as the mean ± SE. SPSS 16.0 (SPSS Inc., Chicago, IL, USA) was used to analyze the data. The Student's *t*-test was employed to assess the statistical differences between the two groups and the one-way ANOVA was conducted for more than two groups. $p < 0.05$ was considered statistically significant.

Results

The PMCs were cultured for 48 hours with high glucose medium. Microscope observation revealed that the control cells were oval, grown adherently, and arranged closely; while after high glucose stimulating, the PMCs became longer, fusiformis, and were arranged in disorder. Real-time PCR reaction amplification curve (see Figure 1) shows that after high glucose stimulating for 48 hours, the expression level of E-cadherin significantly decreased and was 35.6 ± 5.674% of the control group ($p < 0.01$). While the expression level of Vimentin significantly increased and was 2.45 ± 0.245 times than the control ($p < 0.01$). All the results suggested that EMT induced by high glucose in PMCs had genetic changes.

The Relative Expression Levels of miRNAs During EMT

MicroRNAs are involved in regulating the occurrence and development of renal diseases. The microRNAs (miR-193a, miR-21, miR-15a, miR-16 and let-7e) expressions related to kidney development and diseases were assessed during EMT. Relative expression levels were calculated normalizing with U6. The results (Figure 2) showed that compared with control, the relative expression level of miR-193a increased by 2.56 ±

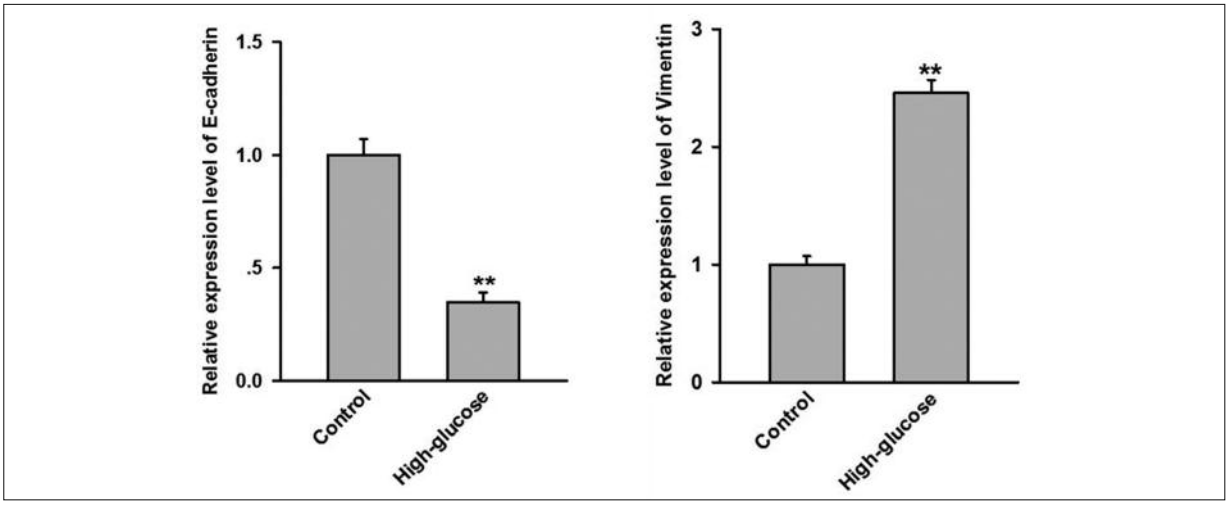

Figure 1. The relative expression levels of genes. Note: $^{**}p < 0.01$ vs control.

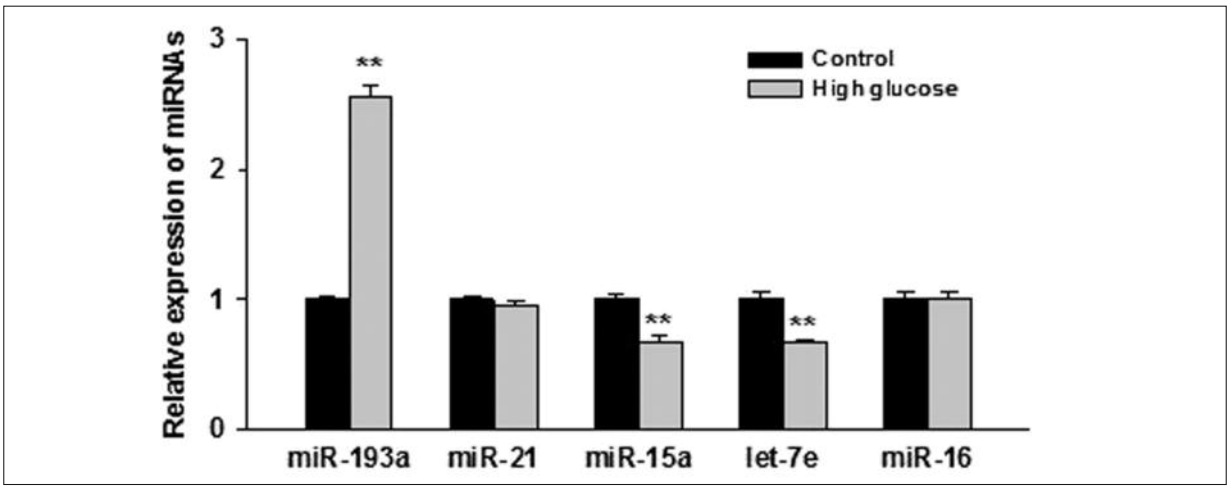

Figure 2. The relative expression levels of miRNAs. Note: $^{**}p < 0.01$ vs control.

0.32 times ($p < 0.01$). While the relative expression levels of miR-15a and let-7e decreased by 67.3 ± 9.53% and 66.5 ± 8.35% respectively ($p < 0.01$), there were no significant changes of miR-16 and miR-21 expressions ($p > 0.05$).

The Expression Pattern of miR-193a During EMT Induced by High Glucose

The expression levels of miR-193a at different times (12 hr, 24 hr, 36 hr and 48 hr) were detected using real-time PCR. The results showed that the expression level of miR-193a increased after stimulating for 12 hours and was correlated with stimulation time (Figure 3). The results indicated that miR-193a might play an important role in the process of EMT induced by high glucose.

Discussion

In this study, after inducing by high-glucose in PMCs, EMT was confirmed by the morphological changes of the cells and the expression changes of the EMT marker genes. Some microRNAs (miR-193a, miR-21, miR-15a, miR-16 and let-7e) expressions related to kidney development and diseases, were assessed by real-time PCR. The expression level of miR-193a increased, and miR-15a and let-7e decreased, while miR-16 and miR-21 had no significant changes. The increase of miR-193a was correlated with stimulus duration, suggesting that miR-193a played an important role.

A microRNA is an endogenous non-coding small RNA molecule. It plays an important modulatory role in a series of life processes, such as cell proliferation, differentiation and apoptosis, embryo development, morphogenesis and diseases[11,12]. Recent studies have found a series of microRNAs associated with EMT[13,14], suggesting that the microRNA may play an important role in peritoneal fibrosis. Gregory et al[15] reported that members of the miR-200 family (miR-200 a, miR-200 b, miR-200 c, miR-141 and miR-429) and miR-205 were own regulated during EMT induced by TGF-β in MDCK cells. Zavadil et al[16] found that 8 microRNAs (miR-21, miR-32, miR-137, miR-346, miR-136, miR-192, miR-210 and miR-211) were related to changes to EMT induced by TGF-β in human skin keratinocytes. These findings suggest that microRNA may play an important regulatory role during in EMT in PMCs.

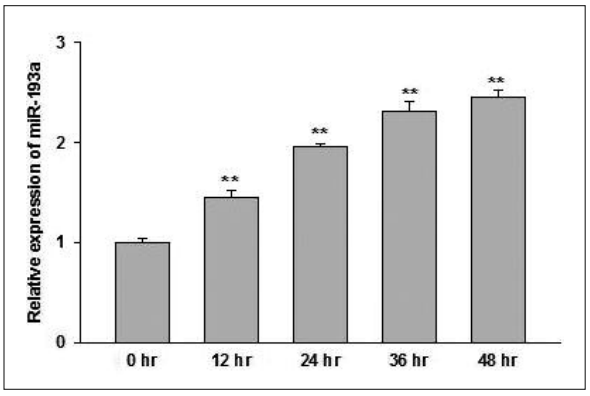

Figure 3. The expression levels of miR-193a at different times induced by high-glucose. Note: $^{**}p < 0.01$ vs the 0 hr group.

MiR-193a is an independent transcription microRNA in the intergenic region on chromosome 17 in humans. Previous studies[17] demonstrated that miR-193a was abnormal in tumor tissue and may regulate the occurrence and the development of the tumor. In acute myelogenous leukemia, miR-193a was found to suppress the tumor through the inhibition of c-kit[17].

A recent study[18] indicated that miR-193a played an important role in the focal segmental glomerulosclerosis. It has not been reported that the miR-193a regulates the peritoneal fibrosis process. However, we found that miR-193a increased during EMT induced by high glucose in PMCs. The preliminary results indicated that the miR-193a might be involved in the EMT of the PMCs and regulates peritoneal fibrosis.

Conclusions

MicroRNAs are very important in kidney-related diseases and can be regarded as new drug targets to treat renal diseases. As a new research field, the regulating effects of microRNA in the disease development are getting more attention. Further studies on the effects of microRNA on diseases and the application of microRNA as a target for new therapeutic interventions are required.

Conflict of Interest

The Authors declare that there are no conflicts of interest.

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