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Influence of Different Glucose Concentrations on the Expression of miR-29c-3p microRNA in Mesenchymal Stem Cells

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ABSTRACT
Background: *miR-29c-3p* manages a set of genes involved in regenerative medicine, and It seems that hyperglycemia in diabetic patients influences the power of stem cells to tissue regeneration the difficulties of diabetes by affecting the expression *miR-29c-3p* in mesenchymal stem cells. The study aims to analyze the effect of various glucose concentrations on the miR*-29c-3p expression* in mesenchymal stem cells.
Materials and Methods: After receiving donated mesenchymal stem cells from Tarbiat Modares University, these cells were cultivated in a DMEM culture medium, including three different concentrations of glucose 250, 140, and 100 mg/dl. RNA was extracted from these cells after 72 hours, the Real-Time PCR technique assessed the expression of miR-29c-3p, and the results were analyzed by REST software.
Results: *miR-29c-3p* expression in cells at concentrations of 140 and 250 mg/dL compared to typical situations (100 mg/dl) was significantly decreased ($P<0.05$), which declined at a concentration of 250 mg/dl was more.
Conclusion: Reduced *miR-29c-3p* expression in mesenchymal stem cells in chronic and mild diabetic situations demonstrated that diabetes might be one of the significant reasons for mesenchymal stem cells' reduced ability to repair tissue damage.

Keywords: Diabetes; Mesenchymal stem cells; miR-29c-3p expression

INTRODUCTION

Mesenchymal stem cells originate from the mesoderm and are capable of self-renewal and multiple differentiation into mesodermal categories such as chondrocytes, osteocytes, adipocytes, and differentiation into ectodermal and endodermal cells[1]. Easy separation of mesenchymal stem cells from adipose tissue, bone marrow, nerve tissue, amniotic fluid, umbilical cord, Wharton jelly, placenta, and dental pulp has led to the treatment of various diseases ranging from genetic disorders to tissue ischemia, diabetes and its complications [2,3]. In addition, other features that make them attractive for the treatment of human diseases are the absence of MHC II and the lack of co-stimulatory molecules such as CD40, CD80, and CD86, which allow

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allogeneic transplantation without suppression of the immune system [4]. Diabetes mellitus is a group of heterogeneous metabolic disorders characterized by hyperglycemia with impaired metabolism of carbohydrates, fats, and proteins, resulting from defects in insulin secretion or function[5]. The global prevalence of diabetes mellitus in 2010 was about 6.8% or 285 million adults (20-79 years old), and this is expected to reach 7.7% or 439 million by 2030, and this increase will be seen in developing countries[6]. In recent years, mesenchymal stem cells have played an essential role in regenerative medicine due to their multipotency and paracrine secretion of angiogenic factors, cytokines, and immune-modulating factors[7]. The behavior of mesenchymal stem cells may change due to various factors and microenvironment. Factors that affect the behavior of these cells include glucose concentration and the expression of various miRNAs in these cells (7-9). miRNAs participated in various physiological and pathological functions such as cell development, differentiation, proliferation, migration, stress responses, angiogenesis, apoptosis, cancer, and diabetes[10].

miR-29c-3p is involved in the aging process of mesenchymal stem cells and also reduces expression in most cancers[9,11]. The *miR-29* family in humans includes *has-miR-29a*, *has-miR-29b-1*, *miR-29b-2*, *has-miR-29c*. The genes encoding *has-miR-29b-1* and *has-miR-29a* are on chromosome 7q32.3, while the genes encoding *miR-29b-2* and *has-miR-29c* are on chromosome 1q32.2, and the coding sequences of each two miRNAs in separate clusters[12]. Studies have shown that the miR-29 family targets at least 16 genes in the extracellular matrix. These genes encode several proteins essential for the physiological and pathological formation of the extracellular matrix and include a large number of isoforms of collagen, laminin 1γ, fibronectin, elastin, matrix metalloproteinase 2, and integrin 1β. Also, a network of genes including *TCL-1, MCL-1, CDK6*, and *YY1* are controlled by the *miR-29* family, which are involved in various tissue repair processes, including regulation of cell proliferation, differentiation, apoptosis, and apoptosis, angiogenesis, etc [12]. These molecules are diagnostic markers with high potential for treating diseases, and their expression changes under the influence of various stimuli, of which glucose concentration is one of these stimuli[8,13]. Given the role of miR-29c-3p in various tissue repair processes, proliferation, and differentiation of mesenchymal stem cells in diabetics, hyperglycemia may influence the miR-29c-3p expression in MSCs on the repair of tissue damage from diabetes. Therefore, the effect of changes in glucose concentration on *miR-29c-3p* expression in mesenchymal stem cells was investigated.

MATERIALS AND METHODS

Donated Umbilical cord-mesenchymal stem cells of Tarbiat Modares University Hematology Department were cultured in three T75 flasks containing + 1% Pen Strep (Sigma) DMEM (Gibco) + 20% FBS (Gibco) and three different concentrations of 250, 140, and 100 mg/dl glucose. They were incubated for 37 hours at 37 ° C, 90% humidity, and 5% Co2. After 72 hours, the cells were detached from the flask with Trypsin (CinaGen), and the total RNA extract was extracted from the cell suspension using RNX-plus solution (CinaGene) according to the company's instructions. The concentration of RNA was measured using a nanodrop, and the integrity was checked with 1% agarose gel. RNAs were stored at -80 ° C until cDNA synthesis.

cDNA synthesis

The cDNA synthesis of microRNA molecules was performed using a Pars Genome Company kit (PARSGENOME.Iran) in a two-step reaction according to the company's instructions. The first step involves adding poly-A and the second step is to add the reverse transcriptase enzyme. The first stage reaction was performed at 37 ° C for 10 minutes, and the second stage reaction was performed at 42 ° C for 60 minutes and 85 ° C for 15 seconds. The concentration and purity of cDNA were measured by light density based on the above descriptions. The cDNAs were stored at -80 °C.

Quantitative Real-Time PCR

The miR-29c-3p expression was measured by the qRT-PCR method and thermocycler (Real-Time PCR ABI 7500-USA). Reactions were performed for miR-29c-3p at 20 µL volume; including SYBR green 10µL,

0.5 µL F primer, 0.5µL R primer, 1µL cDNA, and 8µLNuclease Free water at the following for 95 ° C, annealing at 62 ° C for 20 s, and elongation at 72 ° C for 30 s. The Relative Quantification Pfaffle method, with the help of snRNA U6, was used as a reference gene and internal control to achieve temperatures. Primary denaturation was performed at 95 ° C for 5 minutes, 45 cycles, denaturation at 7 s changes in the expression of the desired genes. NTC (No template control) controls and a control sample that did not undergo reverse transcription (negative control) were also used (Figure 1).

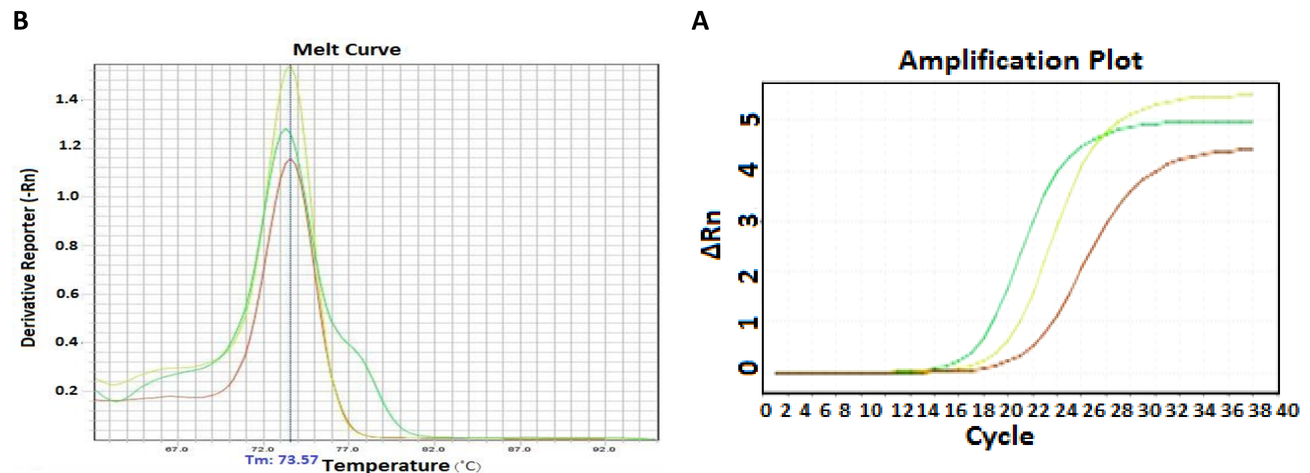

Figure 1. Amplification of miR-29c-3p by qRT-PCR

A) Replication chart of *miR-29c-3p* in cultured UC-MSC at concentrations of 250, 140, and 100 mg/dl glucose,
B) Melting diagram of *miR-29c-3p* amplification product in cultured mesenchymal stem cells at 250, 140, and 100 mg/dl glucose concentrations

RESULTS

Real-time PCR demonstrated that the melting curve of both *miR-29c-3p* and *snRNA u6* genes was obtained as a single peak, indicating the existence of only one PCR product. In addition, the PCR product was loaded on the gel, and it was observed that there was only one specific band in each of the reactions performed with specific primers, which also confirmed the specificity of PCR results in the samples.

Real-time PCR results were analyzed by REST software. In this study, cells cultured at a 100 mg/dl glucose concentration were used as a control sample (similar to normal conditions in terms of blood glucose). The miR-29c-3p expression gene in cells cultured at a concentration of 140 mg/dl (similar Blood glucose in mild diabetic conditions) and a concentration of 250 mg/dl (similar to blood sugar in chronic diabetic conditions) were compared with the control sample (cells cultured at a concentration of 100 mg/dl and similar to standard blood glucose conditions). The results showed that miR-29c-3p expression in cells in chronic and mild diabetic situations (concentration 140-150 mg/dl) compared to cells cultured under normal conditions (concentration 100 mg/dl) compared to the control gene *snRNA u6* decreased significantly (Statistical significance level is $P<0.05$) (Figure 2).

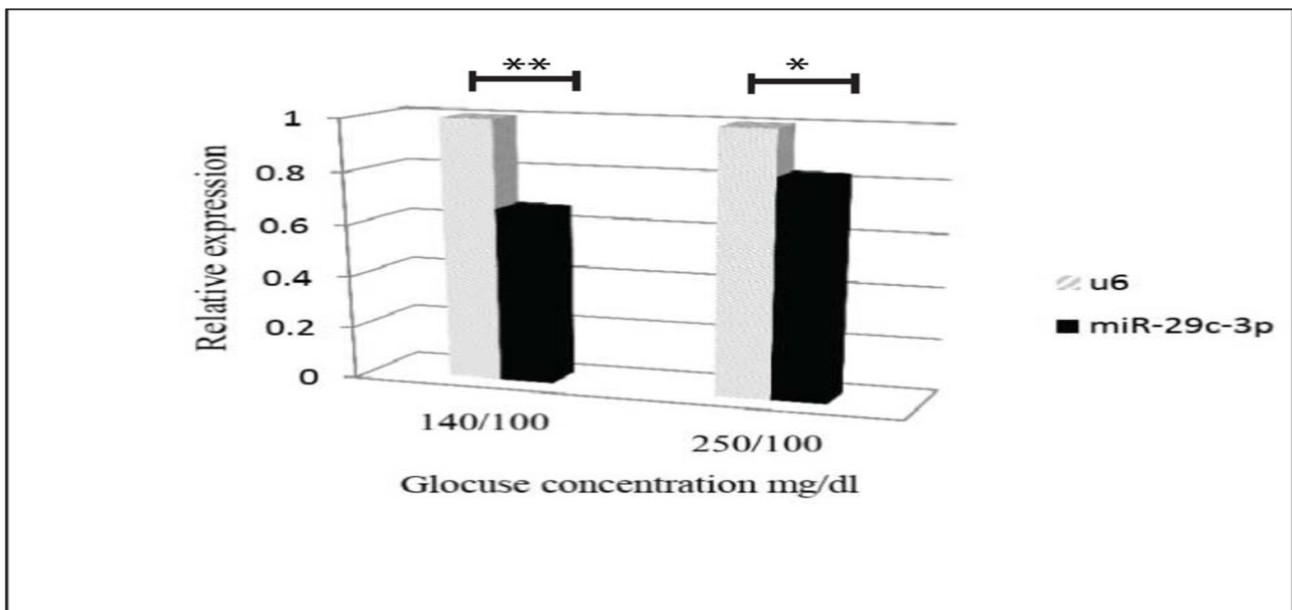

Figure 2. Comparison of *miR-29c-3p* expression compared to *snRNA u6* control gene in cultured cells at mg/dl and glucose compared to cultured cells at mg/dl glucose. (Statistical significance level is P<0.05)

DISCUSSION

In recent years, miRNAs have been identified as a factor in various diseases, including various complications of diabetes [13]. Recently, miRNA-based therapies in various diseases, including atherosclerosis, fibrosis, heart disease, hepatitis C, inflammatory diseases, metabolic disorders, are in clinical trials [14]. miRNAs regulate post-transcriptional gene expression by binding to different target mRNAs and are involved in activating and deactivating different signaling pathways and cellular functions, depending on the type of cellular stimulation [13]. It has been observed that increasing glucose concentration alters the expression of several miRNAs in the tissues of diabetics, including the level of *miR-29c-3p* in insulin target tissues in people with diabetes, including liver[15], skeletal muscle[16], β cells[17] and adipose tissue[16] as well as kidney[18] are affected. Moreover, miR-29c-3p regulates the characteristics of mesenchymal stem cells, including proliferation and differentiation[9]. Wang et al. Showed that the expression of all three members of the miR-29 family (a / b / c) in the proximal tubule and mesenchymal cells of mice and human podocytes is inhibited by TGF-β1. COL1 and COL4 are two targets of miR-29, which are increased by inhibiting miR-29 by TGF-β1, leading to accumulation of extracellular matrix and diabetic nephropathy [19]. In a study by Long et al., MiR-29c was shown to be an essential miRNA in inducing cell apoptosis and extracellular matrix aggregation by targeting Spry1, causing albuminuria and extracellular matrix aggregation in the kidney[18]. Research has also been done on the role of miR-29c-3p in other complications of diabetes, such as diabetic foot ulcers. In research by Haracio et al. On 20 samples of foot skin fibroblasts in non-diabetics and people with non-neuropathic diabetes, the results showed that miR-29c-3p increased in the skin of people with diabetes [20]. There have been several studies on the role of miR-21 in diabetic nephropathy, all of which have shown that miR-21 increases expression in humans and diabetic rats. Research by Nirmalya Dey et al. on human renal mesenchymal cells showed that increased miR-21 expression in these cells led to the targeting of PTEN and decreased its expression, followed by increased AKT phosphorylation, increased mTORC1 activity,

increased fibronectin levels, and COL1α2. And eventually hypertrophy and accumulation of extracellular matrix [21]. Studies have also been done on miR-21 and miR-29 in other complications of diabetes, such as diabetic foot ulcers. In R. Madhyastha's study, comparing the skin tissue of diabetic and healthy mice, it was shown that fourteen miRNAs in diabetic mice had different expressions, with miR-146 and miR-21 being the most significant among the miRNAs studied.miR-21 increased expression 30-fold during routine wound healing in normal mice, whereas its expression was usually higher in the skin of diabetic mice [10]. In the present study, the use of mesenchymal stem cells was considered from two aspects: one as a model of endogenous cells located at the site of diabetic injury in different tissues and the second as a source of exogenous stem cells that can be used to improve diabetic injuries.

In this study, treating these cells with different concentrations of glucose and the effect of glucose on *miR-29c-3p* expression demonstrated that the miR-29c-3p expression in umbilical cord-derived mesenchymal stem cells is reduced in diabetic complications of fibrosis-prone tissues such as the kidney and heart. It seems that this decrease in expression may also have occurred in mesenchymal stem cells located in the kidney and heart, and therefore the mesenchymal stem cells of that tissue are not able to repair the tissue complication caused by diabetes. Decreased miR-29c-3p expression in umbilical cord mesenchymal stem cells by increasing collagen synthesis and extracellular matrix, altering the function of these cells as an exogenous source in the treatment of diabetic fibrous tissues, but concerning diabetic ulcers, miR-29c-3p increases the expression in the skin of diabetics [20]. This increase in expression may not be solely due to glucose; other factors such as the source of MSCs, hypoxia, age, and sex may play a role in increasing miR-29c-3p expression, which contradicts the results of this study.

CONCLUSION

Raised miR-29c-3p expression is involved in diverse tissue repair processes, including cell migration, cell differentiation, proliferation, angiogenesis, and extracellular matrix synthesis and induction of apoptosis. Possibly decreased miR-29c-3p expression in mesenchymal stem cells under hyperglycemic conditions could affect the function of these cells in therapeutic applications for diabetic ulcers and diabetic lesions due to abnormal angiogenesis in diabetic patients and Challenge the treatment strategy. Therefore, miR-29c-3p can be used as a therapeutic target using mesenchymal stem cells to improve the various complications of diabetes. In this regard, the use of these cells can be provided for treatment by modulating the expression of these genes by different strategies in mesenchymal stem cells.

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CONFLICT OF INTEREST

The authors declare that they have no competing interests.

Ethical approval and consent to participate

Not applicable.

Consent for publication:

Not applicable.

Availability of data and materials

Not applicable.

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