



Investigation the Effect of miR-451 and miR-16 in α -Chain on Erythroid Lineage in Electromagnetic Field Residing in Radiated Human Fibroblasts

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Abstract

Background: MicroRNA (miRNA) are endogenous, non-coding, single-stranded short RNA regulatory RNA involved in several biological processes. This study aimed to study the direct differentiation of human fibroblast cells erythrocytes by inducing electromagnetic waves using miR-451 and miR-16 in α chains.

Methods: In the present study, human fibroblasts were radiated with 5, 10, and 15 mT electromagnetic fields. Considering that the best results are obtained with an intensity of 10 mT, this test is performed with 10 mT radiation of the Tesla electromagnetic field. Culturing human fibroblast cells and passing through human fibroblast cells in DMEM (Dulbecco's Modified Eagle Medium) culture medium and then, Counting and determining the percentage of living cells were done. Also, the transaction was performed with PEI (polyethyleneimine) according to the protocol. Then they were divided into 10 study groups. After culturing the radiated cells, determine the differential effect of miR-16 and miR-451 genes in the α chain on human fibroblast cells under 10 MT (Millitesla) static electromagnetic field radiation to the erythroid lineage was examined using the RT - PCR technique.

Results: Our results confirmed that the simultaneous regulation of miR-16 and miR-451 concurrent with the expression of genes involved in the erythroid differentiation pathway with greater potency.

Conclusion: The results showed that the electromagnetic field on the Erythroid lineage of radiated human fibroblasts significantly increased the expression of miR-451 and miR-16. Although this method requires further studies, positive results can be improved. The expression of the studied genes can be suitable for research studies of hematopoietic stem cells.

Keywords: miR-451, miR-16, Erythroid, Fibroblasts, Electromagnetic Field.

Introduction

Endogenous, non-coding, single-stranded short RNAs known as microRNA (miRNA) are involved in several biological processes, such as cell proliferation, differentiation, and apoptosis. They can also serve as tumor suppressor genes or oncogenes [1]. All the main biological processes that control the metabolism and operation of cells and the survival of organisms have been linked to microRNA (miRNA). They control gene expression by base-pairing with complementary target mRNA molecules and

often cause RNA silence [2]. As oncogenes (onco-microRNA) or tumor suppressor molecules, miRNA is frequently discovered to be amplified or down-regulated in cancer cells [3-5]. For instance, miRNA-489 can make cancer cells more susceptible to chemotherapy by inhibiting PI3K/Akt and inducing apoptosis, two biological pathways in cancer progression. The role of miRNA-489 prevents Wnt/-catenin, an oncogenic factor that ensures the growth and aggressiveness of malignancies [6]. In addition, miRNAs play a role in several cellular functions,

including the production of neurotransmitters, insulin secretion, circadian rhythms, and immunological response. Furthermore, miRNA can be controlled by oncogenes or tumor suppressors. MiRNAs can also regulate oncogenic or tumor suppressor pathways [7]. Therefore, the discovery and characterization of the novel onco-suppressor miRNA for specific tumor types will aid in creating new anticancer therapy approaches.

Erythroid progenitor proliferation, terminal erythroid differentiation, and the nucleus, is significantly regulated by a particular miRNA [8]. Additionally, during the differentiation of hESCs into CD34+ hematopoietic cells and subsequent development of the CD34+ cells into the erythroid lineage, expression levels of miR-142-3p, miR-142-5p, miR-146a, and miR-451 were dynamically altered [9]. Moreover, miR-451 and miR-150 expression regulation could be a powerful substitute to stimulate cytokines for CD133+ differentiation into the erythroid lineage. The terminal erythroid differentiation process for erythroblast chromatin condensation and nucleus depends on the controlled expression of several miRNAs [10]. Earlier research has demonstrated that miR-16 plays a unique role in encouraging cardiovascular endothelial damage. Additionally, by targeting IRS1, miR-16 may boost the vitality of cardiomyocytes and the development of vascular endothelial cells [11]. Moreover, miR-16 may impact numerous genes involved in ribosome synthesis, according to a bioinformatics study of its target genes [12]. The erythroid-related phenomena and the tumor-suppressor abilities of miR-16 in cell lines of different origins may be explained by this action. As a potent tumor-suppressor molecule, miR-16 has the potential to be used pharmacologically to treat erythroid-related disorders [2].

Erythropoiesis is a complex process in which a hematopoietic stem cell in the bone marrow environment differentiates into the erythroid class. This cell maturation process leads to reticulocytes' formation in the bone marrow [13]. The primary cytokine in erythropoiesis is erythropoietin, secreted from the kidneys. SCLO, GATA-1, and EKLF-4 are significant transcription factors in this process [14, 15]. Red blood cells have recently been revealed to include miRNA, proving that erythroid differentiation is a complex process and emphasizing the importance of several miRNAs. These chemicals often work by causing an inhibitory influence on the genes of other genes or by removing the inhibitory effect of particular erythroid genes. Many microRNA target each, and one microRNA might interact with hundreds of target mRNA. Due to this, there are many post-transcriptional regulation and activity levels, such as the suppression of microRNA-mediated translation, concurrent protein degradation, and incomplete translation termination. MiRNAs miR-21, miR-144, and miR-451 increased during erythroid line differentiation while miRNA miR-221, miR-155, miR-150, miR-24, miR-222, and miR-223 decreased [16].

Fibroblasts are the main cells located in connective tissue. They are responsible for the synthesis and secretion of connective tissue compounds and precursor molecules of various types of collagen and elastin fibers [17]. Organelles involved in protein synthesis are widely seen in fibroblasts. The secretory program of fibroblasts determines the structure of the extracellular

matrix and thus provides the basis for the construction of a particular type of connective tissue. Fibroblasts play a vital role in the synthesis of collagen and extracellular matrix, as well as in wound healing [18, 19]. Fibroblasts make collagen, glycosaminoglycans (GAGs), reticular, elastic fibers, and glycoproteins in the extracellular matrix and cytokine TSLP [20]. However, unlike epithelial cells, fibroblasts have a perfect ability to migrate individually into the substratum layer [21]. While epithelial cells line the inner surface of the body's organs, fibroblasts and other related connective tissues form the external appearance of living organisms. Rat embryo fibroblasts were often used as nutrient cells in human stem cell research. However, due to the existence of cell culture media with a certain amount of defined nutrients, their use is gradually reduced [22, 23].

In recent years, the use of electromagnetic waves in human life has increased dramatically. A beam is a form of energy emitted in a vacuum or matter. Some types of rays have mass, and others have no mass and have the power to penetrate materials due to energy [24, 25]. Electric and magnetic fields with a frequency of 300 kHz to 300 GHz always exist together and are called electromagnetic fields. Sources and devices that produce radio and microwave radiation are diverse [26]. Exposing the human body to electric and magnetic fields may induce an electric current in different parts of the body. The tissue may absorb some field energy, or both may occur simultaneously [28]. A constant magnetic field, at any point in time, has a constant value over time. A constant magnetic field, often characterized by a quantity of magnetic flux density, occurs around permanent magnets and wires carrying a constant current [29]. Magnetic fields cause eddy currents in the body. The electric field from the transmission lines on the body's surface induces an electric charge [30]. The primary purpose of a radiant system is to generate electromagnetic waves according to the subject under study and includes all radiation parameters and temporal and spatial variability. In addition, radiation systems require specific additional data to eliminate or reduce non-electromagnetic radiation. Irradiation systems should be controlled at different temperatures and provide a suitable environment for tested animals regarding nutrition, respiration, and other necessary conditions. Any samples of the test specimens should not be exposed to chemical or physical agents [26]. In general, this study aimed to determine the differentiation effect of miR-451 and miR-16 in the α chain on human fibroblast cells under 10 mT (Millitesla) static electromagnetic field radiation to the erythroid lineage.

Methods

Electromagnetic field radiation

Exposure to the electromagnetic field after forming the single-celled layer, the cells were treated with 5, 10, and 15 mT (Millitesla electromagnetic fields) for seven consecutive days (radiation subgroup cells). In the laboratory, a machine has been designed and built, causing the creation of an electromagnetic field.

Treatment Procedure

Human fibroblasts were obtained from Bushehr Persian Gulf Biomedical Sciences Research Institute. In this study, human fibroblasts were radiated with an electromagnetic field of 5, 10, and 15 mT (Millitesla electromagnetic field). Considering the results are obtained with an intensity of 10 mT [27], this test is performed by radiating 10 mT and consists of 10 study groups:

1. Control group that was not treated with electromagnetic field (NR-Untreated).
2. Radiation group (R) is treated with 10 mT (R-Untreated).
3. Scramble Group (Sr) (R-Kanamycin).
4. Radiation group (R) and Scramble group (Sr) (R-Kanamycin).
5. Group without radiation and under the influence of miR-451 (NR-miR-451).
6. Group without radiation and under the influence of miR-16 ((NR-miR-16).
7. Non-Radiated and simultaneously affected groups miR-451 and miR-16 (NR-miR-16+451).
8. Radiation group (R) and affected by miR-451 (R-miR-451).
9. Radiation group (R) and affected by miR-16 (R-miR-16).
10. Radiation group (R) and simultaneously affected by miR-451 and miR-16 (R-miR-16+451).

Cell Culture

Human fibroblast cells were melted and passed through human fibroblast cells in DMEM (Dulbecco's Modified Eagle Medium) culture medium. Then, by separating human fibroblast cells and transferring them to 4-well plates, the supernatant culture medium inside the flask was drained, and the bottom cells of the flask were washed twice with PBS. Add 1.25 ml of trypsin to the flask and incubate in an incubator at 37 °C, 5% CO₂ pressure, and 95% humidity for 2 minutes, then place about 10,000 cells in each well and 1 Ml of cell culture medium containing 10% FBS was added. The cells were incubated for 24 hours to form a cell monolayer, after which treatment of the cells would begin. Finally, according to the protocol, we counted and determined the percentage of living cells and transfection with PEI (polyethyleneimine).

Quantitative Real-Time Polymerase Chain Reaction

RNA extraction, RT-PCR, and quantitative RT-PCR assays Total RNAs were extracted from all study groups using TRizol reagent according to the manufacturer's instructions (Yekta Tajhiz Azma, Iran). Complementary DNA was produced with the cDNA Synthesis kit (Yekta Tajhiz Azma, Iran) according to the manufacturer's protocol. Moreover, to check the purity of Total RNA electrophoresis on 2% agarose gel, 5s, 18s, and 28s bands were observed (Figure 1). qRT-PCR reactions were set up and run on a Step-One plus real-time PCR system (ThermoFisher, U.S.A) according to the manufacturer's protocol. Housekeeping genes GAPDH were used in the technique RT-PCR. qRT-PCR (Real-Time Quantitative Reverse Transcription PCR) products were checked by gel electrophoresis according to the product sizes. Primer sequences are given in the supplemental Table 1.

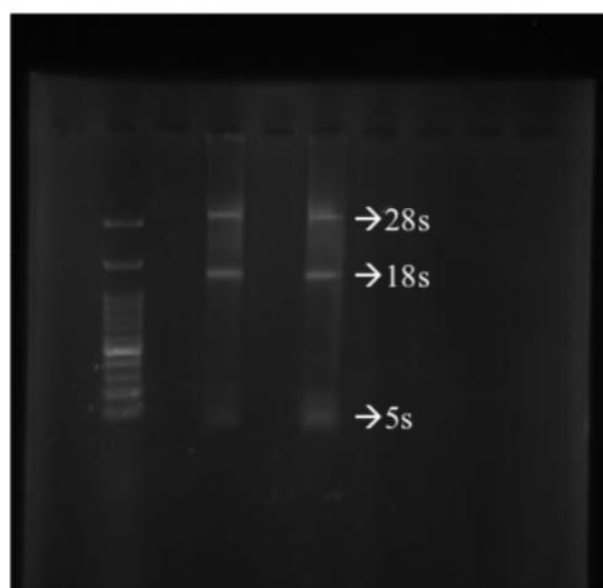


Figure 1. Total RNA electrophoresis on 2% agarose gel. Bands 5s, 18s and 28s are seen in the image.

Table 1. qRT-PCR Primers for α chain

Genes	Sequences 5' → 3'
α chain	Forward: 5' GCTCTGCCAGGTTAAGGG 3' Reverse: 5' CAGTGGCTTAGGAGCTGAAG 3'
GAPDH	Forward: 5' AAGGTGGTGAAGCAGGCG 3' Reverse: 5' AGCGTCAAAGGTGGAGGAG 3'
miR-16	Forward: 5' GGCATAGCAGCACGTA 3' Universal Reverse Primer (100 Reactions) BN-0011.17.5. Stem Cell Technology Research Center, Iran.
miR-451	Forward: 5' CGA GAAACCGTTACCAT 3' Universal Reverse Primer (100 Reactions) BN-0011.17.5. Stem Cell Technology Research Center, Iran.

Statistical Analysis

To find significant differences in the investigated features across the groups, a one-way analysis of variance (ANOVA) was performed. The data were analyzed using GraphPad Prism software version 6. Statistical significance was considered as a P-value of less than 0.001. Also, the normal distribution of the data was evaluated. The experiments were performed three times in duplicate, and data are expressed as mean \pm standard deviation (SD).

Results

α -chain Gene Expression

Based on the One-Way ANOVA test, changes in α chain gene expression in the radiated study groups were also statistically significant ($P < 0.001$). As shown, the high expression level of microRNA-16 was seen in radiated groups, and the expression level of miR-16 and miR-451 was also higher in the radiated groups (Figure 2). Also, changes in α chain gene expression in the study groups were statistically significant ($P < 0.001$) (Figure 2).

Moreover, mRNA of α genes was significantly over-expressed in the concurrent group compared to the two groups compared to the control group (untreated). The group treated with miR-16 showed a more significant increase in expression than those treated with miR-451. Also, the group treated with miR-16 + miR-451 showed a significant increase in expression ($P < 0.001$) (Figure 3).

In general, according to the obtained results, the effect of the magnetic field in 10 mT radiation increases the expression of miR-16 and miR-451 genes (P -value < 0.001) (Figure 4).

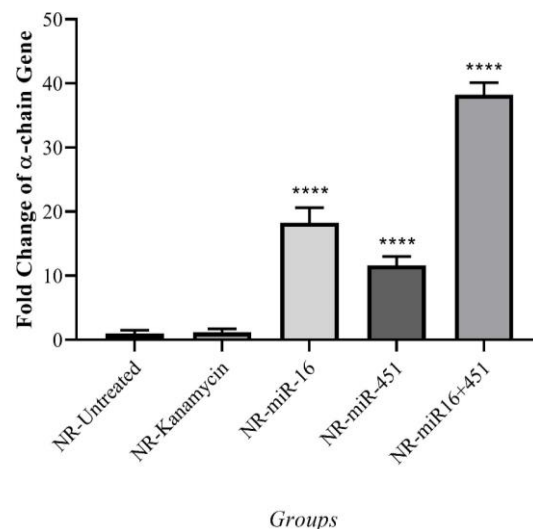


Figure 2. The results of Fold Change of α -chain for miR-16 and miR-451 without radiation. *NR-Untreated: Control group that is not treated with electromagnetic field; NR-Kanamycin: Scramble group (Sr) (Kanamycin antibiotic-resistant group) without radiation; NR-miR-451: Group without radiation and under the influence of miR-451; NR-miR-16: Group without radiation and under the influence of miR-16; NR-miR-16+miR-451: non-radiated and simultaneously affected groups miR-451 and miR-16.

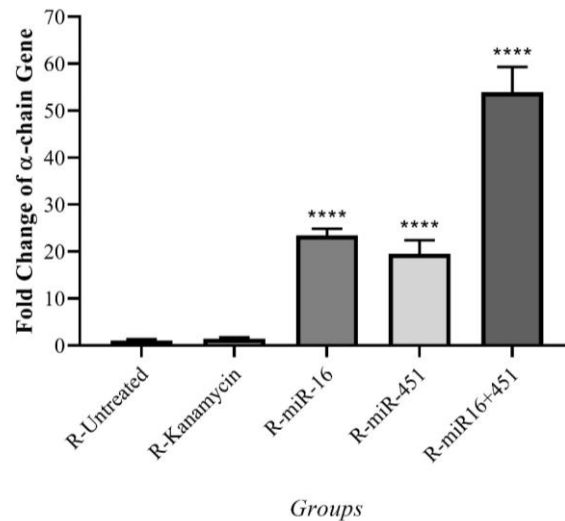


Figure 3. The results of Fold Change of α -chain for miR-16 and miR-451 without radiation. *R-Untreated: Radiation group (R), treated with 10 mT; R-Kanamycin: Radiation (R) and Scramble group (Sr) (Kanamycin antibiotic-resistant group); R-miR-451: Radiation group (R) and affected by miR-451; R-miR-16: Radiation group (R) and affected by miR-16; R-miR-16+miR-451: Radiation group (R) and simultaneously affected by miR-451 and miR-16.

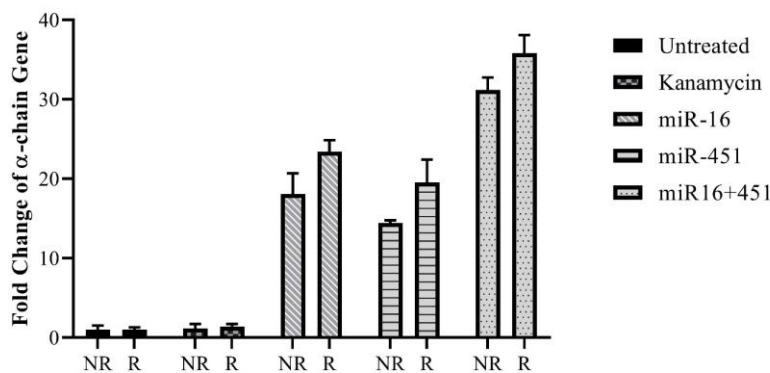


Figure 4. Comparative diagram of radiated and non-radiated samples in α -chain for miR-16 and miR-451 genes. *R: radiated samples; NR: non-radiated samples.

Discussion

MicroRNA (miRNA) is small, non-protein-coding RNA that identify target sites in the 3' untranslated regions (UTRs) of cognate mRNA and either destabilizes or inhibits protein translation. MiRNA has also been found to play a crucial role in hematopoietic lineage differentiation [31, 32]. For example, researchers used microarray technology to do gene-expression profiling and discovered that miR-16 and miR-451 were up-regulated 35-fold and 3-fold, respectively, throughout erythroid development [33].

In a study, it turned into determined that dysregulated expression of microRNAs is related to neoplasia. Additionally, the levels of maturity miR-16 are abnormally improved in CD34(+) cells of patients with miR-16-1 polycythemia on chromosome 13. This information recommends that the deregulation of miR-16-2 contributes to the strange expansion of the erythroid lineage in polycythemia. However, the mechanisms of miR-16-2 overexpression have now no longer been elucidated, as no genetic abnormalities had been detected on the miR-16-2 locus [34]. In addition, another study confirmed that microRNA-451 (miR-451) regulates erythropoiesis in vivo. Mice lacking miR-451 show decreased hematocrit, erythroid differentiation defects, and ineffective erythropoiesis in response to oxidative stress [35]. Collectively, these results of the current study confirmed that simultaneous regulation of miR-16 and miR-451 stimulates the expression of genes involved in the erythroid differentiation pathway more potently. However, the mechanisms of miR-16-2 overexpression have not yet been elucidated, as no genetic abnormalities were detected at the miR-16-2 locus. It is suggested to conduct more extensive research on the mechanism of the discriminatory effect

of miR-16 and miR-451.

This study aimed to evaluate if the α -chain in miR-16 and miR-451 may function in the erythroid lineage in the electromagnetic field residing in radiated human fibroblasts. We evaluated how high levels of these miRNAs affected erythroid-dwelling fibroblast development. RT-PCR results revealed that when cells were treated with kanamycin, miR-451 erythroid level indicators were up-regulated, and miR-451 over-expression increased hemoglobin expression. The gene's mRNA was considerably over-expressed in the concurrent group. Compared to the untreated control group, the group treated with miR-16 exhibited a markedly higher expression than those treated with miR-451. Furthermore, the group treated with miR-16 + miR-451 had a considerable increase in expression. These findings revealed that concurrent regulation of miR-16 and miR-451 increases the expression of genes involved in the erythroid development pathway. However, the targets of miR-451 in humans have to be discovered.

Conclusion

Our findings show that enhanced miR-16 and miR-451 expression may play a critical role in erythroid differentiation for in vitro mesenchyme stem cells in vitro and synthetic erythrocyte production in the lack of concurrent cytokines. Since the essential issue for individuals with hemoglobinopathies such as sickle cell anemia and Thalassemia is a failure to form adult globin (HbA), reactivation of alpha and beta-globin chains has been necessary to save mice's lives in Thalassemia (alpha and beta). The expression of miR-16, miR-451, and other miRNAs may be necessary for developing successful gene therapy treatment options to correct these disorders. With these discoveries, we will better comprehend how miRNAs act on embryonic stem cells, improve the differentiation of erythroid cell lineage, and create artificial RBCs without the need for any stimulator cytokines. However, the entire in vitro erythropoiesis employing miRNA expression regulation must be proven functionally and visually for clinical applications. Therefore, the current study represents the first step in this sector, and more research is required.

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Conflict of Interest: The authors declare no conflict of interest in this study.

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Ethical statement: This study is experimental research approved as a thesis with an Ethical code number of "IR.BPUMS.REC.1400.030" in Bushehr University of Medical Sciences.

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