Karoubi et al., *BioImpacts.* 2024;14(1):27817
doi: 10.34172/bi.2023.27817
http://bi.tbzmed.ac.ir/

Static electromagnetic field and recombinant human fibroblasts encoding miR-451 and miR-16 increased cell trans-differentiation to CD^{71+} and CD^{235a+} erythroid like progenitor

Nafiseh Karoubi[1], Gholamreza Khamisipour[1,2*], Nahid Babaei[1], Narges Obeidi[1,3], Abbas Doosti[1,4]

[1]Department of Cell Biology and Genetics, Bushehr Branch, Islamic Azad University, Bushehr, Iran
[2]Department of Hematology, Faculty of Allied Medicine, Bushehr University of Medical Sciences, Bushehr, Iran
[3]Department of Hematology, Bushehr University of Medical Sciences, Bushehr, Iran
[4]Biotechnology Research Center, Shahrekord Branch, Islamic Azad University, Shahrekord, Iran

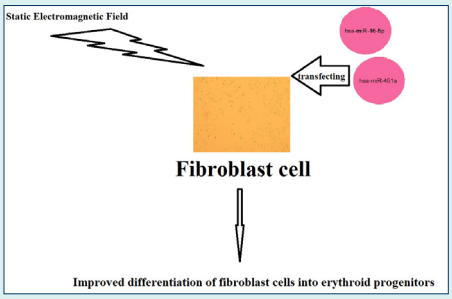
Article Info

Article Type:
Original Article

Article History:
Received: 15 Feb. 2023
Revised: 10 Jun. 2023
Accepted: 18 Jun. 2023
ePublished: 8 Aug. 2023

Keywords:
ELF-EMF
Erythroid
Fibroblast
Gene
Hematopoiesis

Abstract



Introduction: *Ex vivo* blood production is an urgent need of most countries, and creating production protocols can save the lives of many patients. Despite the recent advances in blood production in *ex vivo* conditions, its high-scale production is not yet possible, and requires further studies. Therefore, by transfecting fibroblast cells with miR-16, and miR-451 genes, as well as applying low frequency electromagnetic fields (ELF-EMF) treatment, we tried to increase the differentiation of these cells into CD^{71+} and CD^{235a+} erythroid like progenitors.

Methods: After preparation, and cultivation of human dermal transgenic fibroblast cells, they were transfected by Plenti3-hsa-miR451, Plenti3-hsa-miR16 and Plenti3-backbone inserted into *E. coli* Stbl4 genome. Then, transgenic fibroblast cells were treated with 10mT ELF-EMF every day for 20 minutes for 7 days. Using a flow cytometer, the expressions of CD^{71}, and CD^{235a} were studied in these cells, and the expressions of genes involved in hematopoiesis were studied using the RT-PCR technique.

Results: The results indicated an increase in the differentiation of fibroblast cells treated with 10mT ELF-EMF to erythroid like progenitors. Furthermore, the percentage of CD^{71+} and CD^{235a+} cells was the highest in irradiated cells encoding miR-16 and miR-451, which indicates their differentiation into erythroid like progenitors. Also, in the transgenic cells treated with ELF-EMF, an increase in the expressions of *α-chain*, *β-chain*, *γ-chain* and *GATA1* genes was observed, which indicates the potential of these cells for hematopoiesis. However, there was no significant difference in the expression of *CD34* and *CD38* genes in these cell lines.

Conclusion: Both ELF-EMF and upregulations of miR-16 and miR-451 lead to improved differentiation of fibroblast cells into erythroid like progenitors.

Introduction

Blood production in *ex vivo* conditions to sustainably meet the needs of different patients can reduce treatment problems.[1] Meanwhile, one of the good sources for this purpose is the bone marrow, where it differentiates from the hematopoietic stem cell into erythroid cells during a process called erythropoiesis.[2] The differentiation of erythroid cells from fibroblast cells was reported in many studies.[3] Also, fibroblast cells are very important in the treatment of various diseases in terms of their ability to transform into other vital cells, such as cardiomyocytes, erythroid progenitors, liver cells, etc.[4-6] Thus, the ability of fibroblast cells to differentiate into erythroid progenitors may be used in the *ex vivo* synthesis of human red blood cells. However, despite the positive potential of these cell lines, red blood cell production on a big scale still has a lot of issues, necessitating the improvement of procedures and research into the impact of numerous

*Corresponding author: Gholamreza Khamisipour, Email: ghr.khamisi@gmail.com

variables. One of the important regulatory factors of various cellular processes, including erythropoiesis, are microRNAs, which consist of 18-25 nucleotide sequences. Many studies have emphasized the role of miR-451, and miR-16 in erythroid differentiation. For example, Kouhkan et al reported that the upregulation of miR-451 can induce erythroid differentiation of CD+133 cells.[7] Thus, Papagiannopoulos et al showed that miR-16-5p causes erythroid differentiation of Erythroleukemia cells[8] and the action mechanism can be attributed to the regulation of ribosome biogenesis[8] and inhibiting GATA-1 transcription factor.[9,10]

Low frequency electromagnetic fields (ELF-EMF), which were proven to alter cell proliferation and even death, are one of the elements influencing the differentiation of cells.[11,12] These waves can enter tissues deeply and exert their effects. ELF-EMF can lead to DNA damage, and carcinogenesis.[13] However, there were reports of its therapeutic effects such as wound healing.[14,15] Meanwhile, the duration of ELF-EMF application seems to play an important role in its biological effects.[16] Many studies showed that ELF-EMF has the ability to induce differentiation in different types of cell lines. For example, Chen et al reported that K562 cells were able to differentiate into erythroid cells under ELF-EMF irradiation.[17] Also, Garip-İnhan et al showed that ELF-EMF application every day for 1 hour improves the differentiation of K562 cells compared to applying electromagnetic field at once.[18] Therefore, ELF-EMF has the potential to be used in inducing the differentiation of different cell lines.

The current study aimed to explore the effects of static electromagnetic field and the introduction of miR-451 and miR-16 in human fibroblasts on its cell differentiation to an erythroid like progenitor because it is currently not possible to produce blood on a large scale in *ex vivo* to meet the needs of patients.

Materials and Methods

Fibroblast cell preparation and culture

Human dermal fibroblast cells were purchased from Bon Yakhte Corp. (Tehran, Iran). First, the cells were examined for CD^{34}, CD^{45}, CD^{73}, CD^{90} and CD^{105} biomarkers expressions by flowcytometry. 4 mL of high glucose content (4.5 g/L) DMEM medium (Biosera, France) containing 10% FBS (Gibco, USA) and 1% antibiotic-antimycotic solution (Biosera, France) were used for cell culture. The culture medium in the Falcon was filled with the cell suspension, which was then carefully pipetted into the container. The cell-containing tube was then spun at 230 g for 5 minutes (VISION SCIENTIFIC, South Korea). After removing the supernatant, the cell sediment was suspended in 1 mL of culture medium, and transferred to a 25 cm cell culture flask (SPL, South Korea) containing 4 mL of complete culture medium. After observing the cells inside the flask under an inverted microscope (LaboMed, USA), the flask was transferred to an incubator (MEMMERT, Italy) with a temperature of 37 °C, 5% CO2, and 95% relative humidity (RH).

The percentage of viable cells was determined by cell staining with trypan blue. For this purpose, at first 1 mL cell suspension was prepared, and 20 µL of trypan blue 0.25% was added to cell suspension and poured into a well of a 96-well plate. About 10 µL of the mixture was placed on Neubauer slide and transferred under the microscope.

Transfection of fibroblast cells

To transfer Plenti3-hsa-miR451, Plenti3-hsa-miR16 and Plenti3-backbon (Bon Yakhte, Iran) recombinant plasmids to bacterial cells (*E. coli* Stbl4), 150 µL of Plenti3-hsa-miR451, Plenti3-hsa -miR16 and Plenti3-backbone recombinant plasmids were mixed in 5 mL of Lysogeny Broth (LB) medium, and 3 µL of Kanamycin Sulfate (DNA Biotech, Iran) was added. As a control, a sample made up of 3 µL of kanamycin and 5 mL of LB culture media was used. All samples were then put in a shaker incubator for 24 hours at 37 °C. Amplified (cloned) plasmids were extracted using a plasmid extraction kit from Yekta Tajhiz Azma Corp. (FAPDE050, Iran) based on manufacture instructions.

Transgenic fibroblast cell irradiation

The magnetic field generator shown in Fig. 1 was used to apply the magnetic field. This device consisted of three coiled columns, two ends of each column's wires were connected to the appropriate voltage to apply the magnetic field. A rectifier device, which was an AC to DC current converter, was used to apply the static magnetic field. A VARIAC Variable Transformer was also used to modify the output field while adjusting the input current. The intensity of the field produced by the field generator was measured using a Gaussmeter probe.

In the preliminary test, human fibroblast cells were exposed to electromagnetic field radiation with intensity of 5, 10 and 15 mT. Considering the best result was obtained from the intensity of 10 mT, therefore the test was performed under the radiation of 10 mT static electromagnetic field.

Fig. 1. Magnetic field generator, rectifier and a VARIAC Variable Transformer used in current study to apply static magnetic field on Fibroblast cells.

The treatments were as follows:

1. The control group without electromagnetic field treatment
2. Radiation group (R), treated with 10mT electromagnetic field 20 minutes every day for 7 days.
3. Scramble group (Sr) (Kanamycin)
4. Radiation group (R) and Scramble group (Sr)
5. Recombinant miR-451 cells without irradiation
6. Recombinant miR-16 cells without irradiation
7. Recombinant miR-451 and miR-16 cells without irradiation
8. Recombinant miR-451 cells with 10 mT ELF-EMF irradiation
9. Recombinant miR-16 cells with 10 mT ELF-EMF irradiation
10. Recombinant miR-451 and miR-16 cells with 10 mT ELF-EMF irradiation

CD^{71} and CD^{235a} expressions

After the treatment of fibroblast cells with static electromagnetic field for 7 consecutive days (every day 20 minutes), on the 21st day, fibroblast cells were examined in terms of the expressions of CD^{71} and CD^{235a} cell surface indicators. For this purpose, the colonies were transferred to sterile falcons and centrifuged for 5 minutes at 230 g. After draining the culture media, 500 L of the trypsin enzyme was added, and it was then incubated for 2 minutes in an incubator at 37 °C, 5% CO2, and 95% RH. The cell suspension was then put into tubes for flow cytometry and centrifuged at 230 g for 5 minutes. After centrifugation, the supernatant was drained and 1 µL of CD^{71} and CD^{235a} antibody (Glycophorin A, BioLegend, USA) was added to the tubes, and placed in a dark at 4 °C for 30 minutes. The cells were centrifuged three times with 400 g for 5 minutes, and then, 1000 µL of cold 1× phosphate buffered saline (PBS) buffer was added to each tube to suspend the cells, and the cells were analyzed with a flow cytometry device (BD, USA). In each group, 1×10^5 cells were analyzed and the experiment was repeated three times.

RNA extraction and cDNA synthesis

RNA extraction of transgenic human fibroblast cells was performed using phenol/chloroform method.[19] In brief, 1000 µL of Triazole (Sigma, USA, CAT# 288-88-0) and 200 µL of chloroform were added to cell suspension and centrifuged for 10 minutes at 12000 g at 4 °C. The supernatant was removed and 400 µL of cold isopropanol was added and again centrifuged for 10 minutes at 12 000 g at 4 °C. Precipitated RNA was dissolved in 1000 µL of 75% ethanol and vortexed. It was then centrifuged at 12000 g for 10 minutes at 4 °C. The last step was to add 20 to 50 L of DEPC-treated water. Using a Nanodrop instrument (Denovix, South Korea) and 2 percent agarose gel electrophoresis, respectively, the purity and quality of the collected RNA were verified. To synthesize cDNA, a cDNA synthesis kit (Yekta Tehiz Azma Company, Iran, YT4500) was used. All manufacturer's instructions were followed.

Primer design

The sequences of *α-chain* (Gene ID: 2243), *β-chain* (Gene ID: 3043), *γ-chain* (Gene ID: 2266), *GATA-1* (Gene ID: 2623), *CD*[34] (Gene ID: 947) and *CD*[38] (Gene ID: 952) genes was extracted from NCBI database and the corresponding primers were designed using Gene Runner software. After designing, using NCBI site tool, the sequence of the primers was blasted (https://www.ncbi.nlm.nih.gov/tools/primer-blast/) with the whole human genome and the specificity of the primers for their complementary regions was fully assured. The primers were made by Bon Yakhte Corp. (Iran). GAPDH gene was used as a reference gene (Table 1).

RT-PCR

The reaction mixture included 7.5 µL of RealQ Plus 2x Master Mix Green High Rox (Add bio, South Korea), 1 µL of forward primer, 1 µL of reverse primer, 1 µL of cDNA, 4.5 µL of distilled water, with a final volume of 15 µL. RT-PCR thermal program was set at 1 cycle of 10 minutes at 95 °C, 40 cycles of 30 seconds at 95 °C and 60 seconds at 60 °C (for annealing), and final cycle of 55-95° for melting curve.

Statistical analysis

Two-way analysis of variance (ANOVA) and Tukey's multiple comparison test ($P<0.05$) were used for data analysis. GraphPad Prism V.8 was used for data analysis. Moreover, *t* test was used as a parametric method to compare paired groups. Three repetitions were considered for each group of current study.

Results

Confirmation of fibroblast cell transfection

Green fluorescent protein (GFP) gene expression was seen in transfected fibroblast cells expressing miR-16 and miR-451 under a fluorescent microscope, indicating the validity of their transfection (Fig. 2).

Microscopic examination

During the present study, after one day, the irradiated cells were transferred to DMEM culture medium, and observed with optical microscopy (10X). As can be seen in the Fig. 3, the fibroblast cells changed from spindle-shaped, and adherent to round, single and non-sticky ones.

Cells exposed to 10 mT ELF-EMF radiation were observed after 7, 14, and 21 days, and the findings revealed that the cells had proliferated and formed colonies. Microscopic scans revealed that a number of undifferentiated fibroblast cells were adhered to the culture surface in addition to the proliferating colonies (Fig. 3).

Table 1. The sequences of primers used in current study to amplified the studied genes

Genes	5' → 3' primer sequences	GC%
α chain	Product Length: 160 Forward Primer: 5′ GCTCTGCCCAGGTTAAGGG 3′ Reverse Primer: 5′ CAGTGGCTTAGGAGCTTGAAG 3′	 63.16 52.38
β chain	Product Length: 152 Forward Primer: 5′ CACCTTTGCCACACTGAGTGAG 3′ Reverse Primer: 5′ CCACTTTCTGATAGGCAGCCTG 3′	 54.55 54.55
γ chain	Product Length: 185 Forward Primer: 5′ AACTTCAAACTCTTGGGTAATG 3′ Reverse Primer: 5′ GGAGGCATAGCGGACAC 3′	 63.36 64.71
GATA-1	Product Length: 88 Forward Primer: 5′ CTGTCCCCAATAGTGCTTATGG 3′ Reverse Primer: 5′ GAATAGGCTGCTGAATTGAGGG 3′	 50.00 50.00
CD^{34}	Product Length: 185 Forward Primer: 5′ CTACAACACCTAGTACCCTTGGA 3′ Reverse Primer: 5′ GGTGAACACTGTGCTGATTACA	 47.83 45.45
CD^{38}	Product Length: 118 Forward Primer: 5′ AGACTGCCAAAGTGTATGGGA 3′ Reverse Primer: 5′ GCAAGGTACGGTCTGAGTTCC 3′	 47.62 57.14
GAPDH	Product Length: 114 Forward Primer: 5′ AAGGTGGTGAAGCAGGCG 3′ Reverse Primer: 5′ AGCGTCAAAGGTGGAGGAG 3′	 61.11 57.89

CD^{71+} and CD^{235a+} cell identification

In 10mT ELF-EMF-treated and untreated cells, cells harboring plasmids encoding miR-16 and miR-451, as well as both (miR-16 & miR-451) resulted in a significant enhancement of their differentiation into erythroid like cells (CD^{71+} & CD^{235a+}) compared to control (Fig. 4A, P<0.0001). However, when these cells were treated with 10 mT ELF-EMF for 20 minutes every day for 7 days, the percentage of differentiation of fibroblast cells to erythroid like cells increased by ~20% compared non-treated one (P=0.006, Fig. 4B), indicating that ELF-EMF and transgenic cells encoding miR-16 and miR-451 and both of them significantly improved the differentiation of fibroblast cells into erythroid like cells. Nevertheless, the mean comparison using t test showed that when the cells were exposed to ELF-EMF in all groups, higher percentages

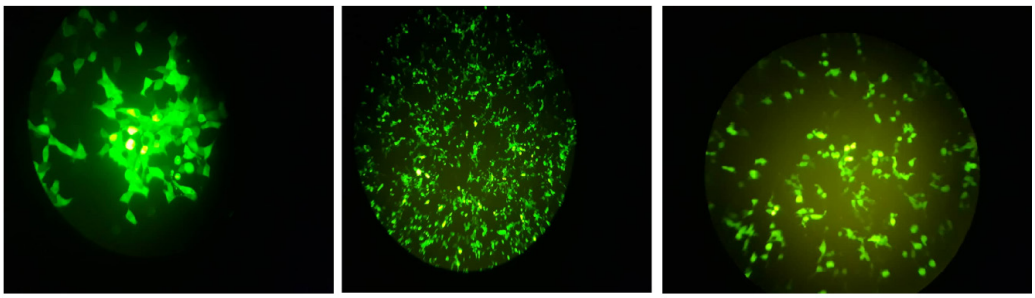


Fig. 2. The expressions of green fluorescent protein (GFP) in transgenic fibroblast cells seen under fluorescent microscope

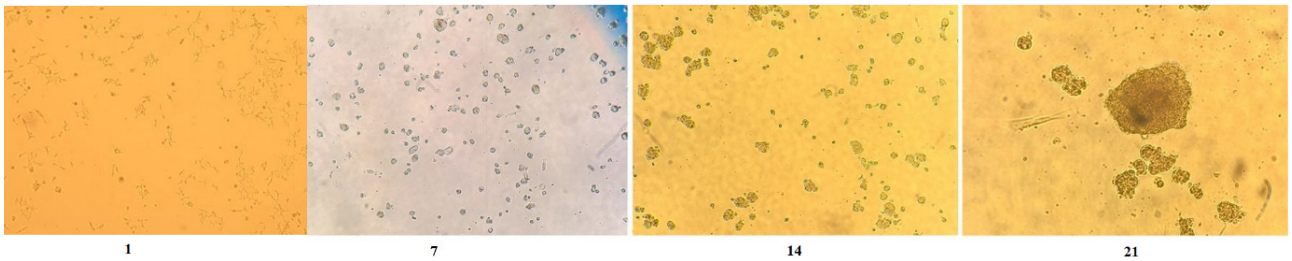


Fig. 3. The human fibroblast cells treated with 10 mT ELF-EMF irradiation on days 1, 7, 14 and 21 evaluated under optical microscopy (10X). The cells exposed to ELF-EMF irradiation 20 min every day for 7 days.

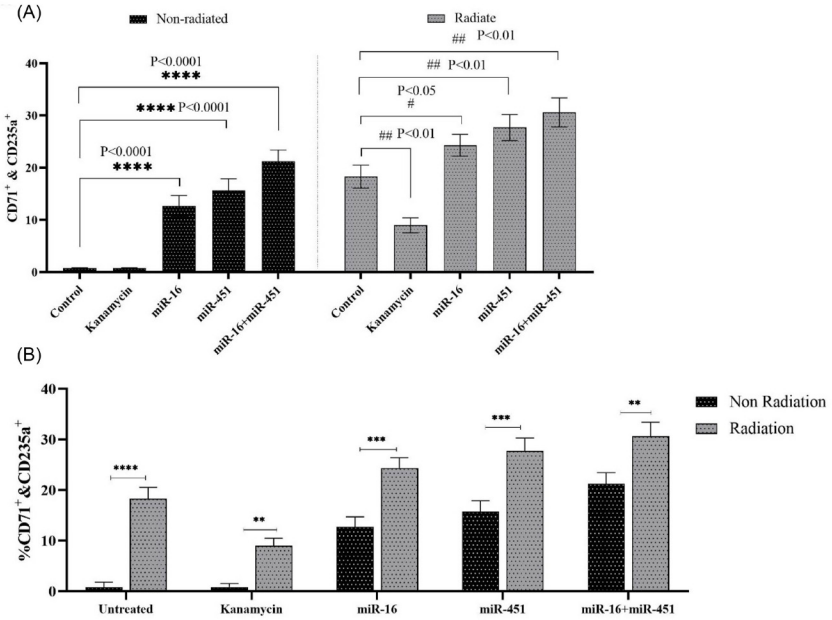


Fig. 4. The percentage of CD^{71+}&CD^{235a+} cells in recombinant miR-16 and miR-451 coding fibroblast cell with or without 10mT ELF-EMF irradiation treatment. (A) Analysis by ANOVA; (B) Analysis by *t* test. The transgenic fibroblast cells were treated with 10mT electromagnetic field 20 min every day for 7 days (n=3).

of fibroblast to erythroid like cell differentiation seen (Fig. 4B). For instance, a comparison of the percentage of cells treated with ELF-EMF and transfected with miR-16 with their control cells showed a substantial increase of almost 50% in the differentiation of cells into erythroid-like cells (P=0.0002). Therefore, transgenic fibroblast cells encoding miR-451 and exposed to ELF-EMF showed an approximately 45% increase in cell differentiation into the erythroid-like lineage compared to their counterparts not treated with electromagnetic field (P=0.0006). The related histograms are shown in Fig. 5.

Genetical analysis

α-chain, β-chain and γ-chain gene expressions

The expressions of all three *α-chain*, *β-chain* and *γ-chain* genes were increased in transgenic fibroblast cells encoding miR-16, miR-451, and miR-16+miR-451 and not treated with ELF-EMF compared to the control. Moreover, in non-transgenic fibroblast cells exposed to ELF-EMF, a significant increase in *a-chain* gene expression was seen compared to the control. When compared to transgenic cells encoding miR-451, this gene's average expression in transgenic cells encoding both miR-16 and miR-451 showed a significant difference (P=0.039), and its expression in these cells was significantly higher than in transgenic cells encoding miR-451 (P=0.006, Fig. 6A). Nevertheless, the exposure of transgenic fibroblast cells encoding miR-16, miR-451 and miR-16+miR-451 to ELF-EMF resulted in significant changes in *a-chain* gene expression compared to the control exposed to ELF-EMF

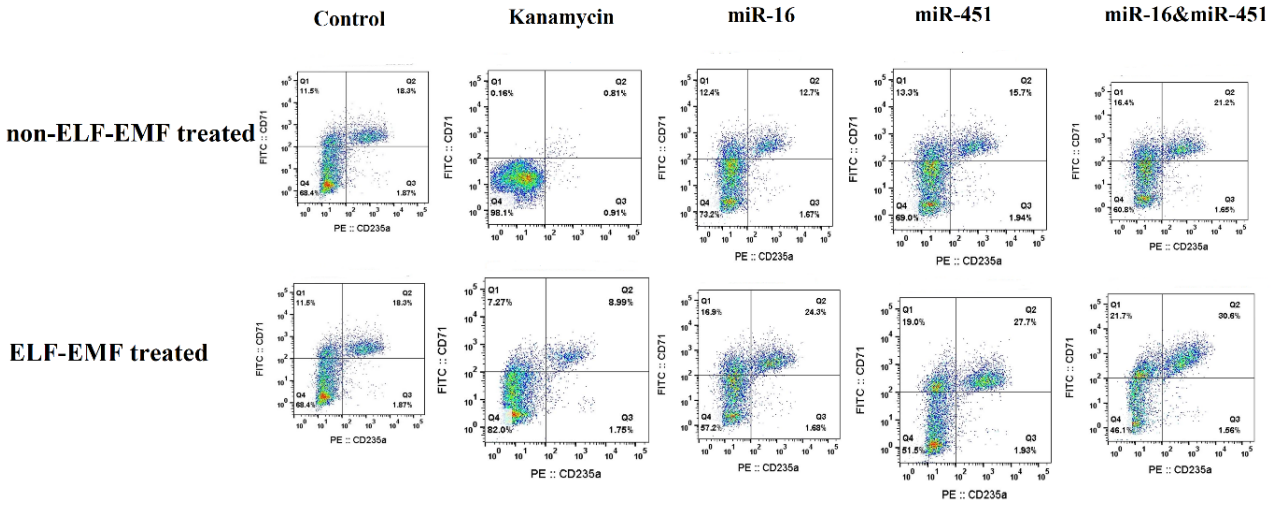


Fig. 5. The flowcytometry histograms for transgenic fibroblast cell with or without 10mT ELF-EMF treatment.

(*P*<0.0001). However, the expressions of b-chain (Fig. 6B), and γ-chain (Fig. 6C) genes in transgenic fibroblast cells encoding miR-16 (*P*<0.0001), miR-451 (*P*<0.0001) and miR-16+miR-451 (*P*<0.0001) and exposed to ELF-EMF increased significantly compared to the control exposed with ELF-EMF.

GATA1, CD^{34} *and CD*^{38} *gene expressions*

GATA1 gene expression in fibroblast cells treated with ELF-EMF and untreated in transgenic cells encoding miR-16, miR-451, and miR-16+miR-451 showed a significant increase compared to the control. Additionally, both cells exposed to ELF-EMF and untreated cells significantly increased in GATA1 gene expression when transgenic cells producing miR-16+miR-451 were compared to transgenic cells encoding miR-16. However, in terms of the expressions of *CD*^{34} and *CD*^{38} genes, no significant differences were seen among different groups of fibroblast cells (Fig. 7).

Discussion

Until today, many studies were conducted to produce red blood cells from hematopoietic stem cells using internal and external factors including transcription factors, growth factors, stimulating cytokines.[20-22] However, these methods are not cost-effective in therapeutic and clinical settings because of their high prices, poor effectiveness, and restrictions on the creation, supply, and upkeep of blood cells.[23] The flexibility of fibroblast cells and the success in transforming them into other types of cells lead to efforts to produce HSC from these cells as an alternative strategy to stem cell-based methods,[24] for this reason in this study transgenic fibroblast cells were used. Therefore, this study was conducted to investigate the induction effect of ELF-EMF and the proven effect of miR-451 and miR-16 in erythropoiesis for the direct differentiation of human fibroblast cells into erythrocyte-like progenitor. The results of the present study showed that 10mT ELF-EMF and transgenic fibroblast cells encoding miR-16,

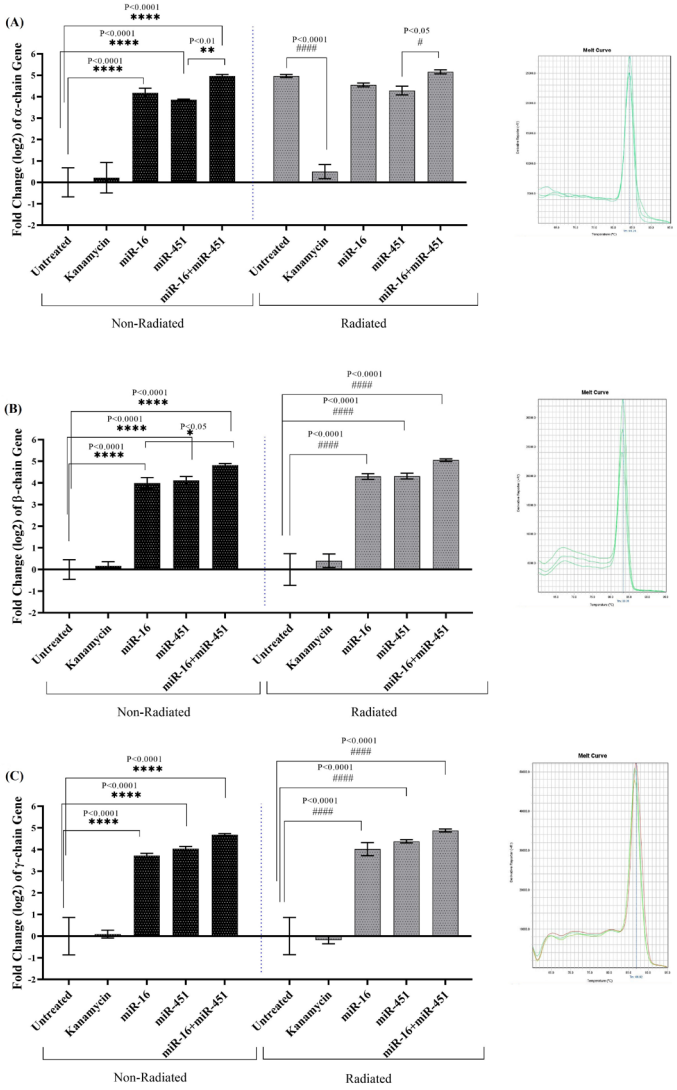


Fig. 6. The expressions of *α-chain* (A), *β-chain* (B) and *γ-chain* (C) genes (Mean±SEM) in non-radiated, and ELF-EMF radiated fibroblast cells transfused with miR-16, miR-451 and miR-16+ miR-451. The melting curves obtained by RT-PCR are showed in right. The transgenic fibroblast cells were treated with 10mT electromagnetic field 20min every day for 7days (n=3).

miR-451 and miR-16+miR-451 lead to the improvement of differentiation of fibroblast cells to erythroid like ones. Thus, the erythroid like lineage target genes α-chain, β-chain, γ-chain and GATA1 overexpressed in the transgenic fibroblast cells encoding miR-16, miR-451 and miR-16+miR-451, which indicates improved differentiation to the erythroid like cells.

Genes involved in hemoglobin production (α-chain, β-chain and γ-chain) in transgenic fibroblast cells encoding miR-16, miR-451 and miR-16+miR-451 significantly overexpressed compared to the control. Nevertheless, no significant difference was seen in the expressions of CD^{34} and CD^{38} genes in different cell groups, which probably indicates the entry of fibroblast cells into the late proerythroblast stage.[25] Moreover, because the fibroblast cells encoding miR-16, miR-451 and both of them led to more CD^{71} expression than $CD,^{235a}$ and considering that the latter is expressed in red blood cells but the former is not expressed, therefore it can be stated that fibroblast cells have differentiated towards erythroid progenitors and have not progressed towards mature cells.[26]

As mentioned, the upregulations of miR-451 and miR-16 in the present study led to increased differentiation of human fibroblast cells to the erythroid like lineage, which is in accordance with the findings of other studies. As an example, Bruchova-Votavova et al demonstrated that up-regulation of miR-451 and miR-150 in K562 cells improved their ability to differentiate into erythroid cells.[9] The result of another study showed that the upregulation of miR-451, but not miR-16, can induce the expression of α, β, and γ globin genes in CD^{133+} cells, and CD^{71} and CD^{235a} were strongly correlated in these cells. Also, miR-451 strongly caused erythroid like differentiation and maturation of CD^{133+} stem cells.[7] The role of miR-

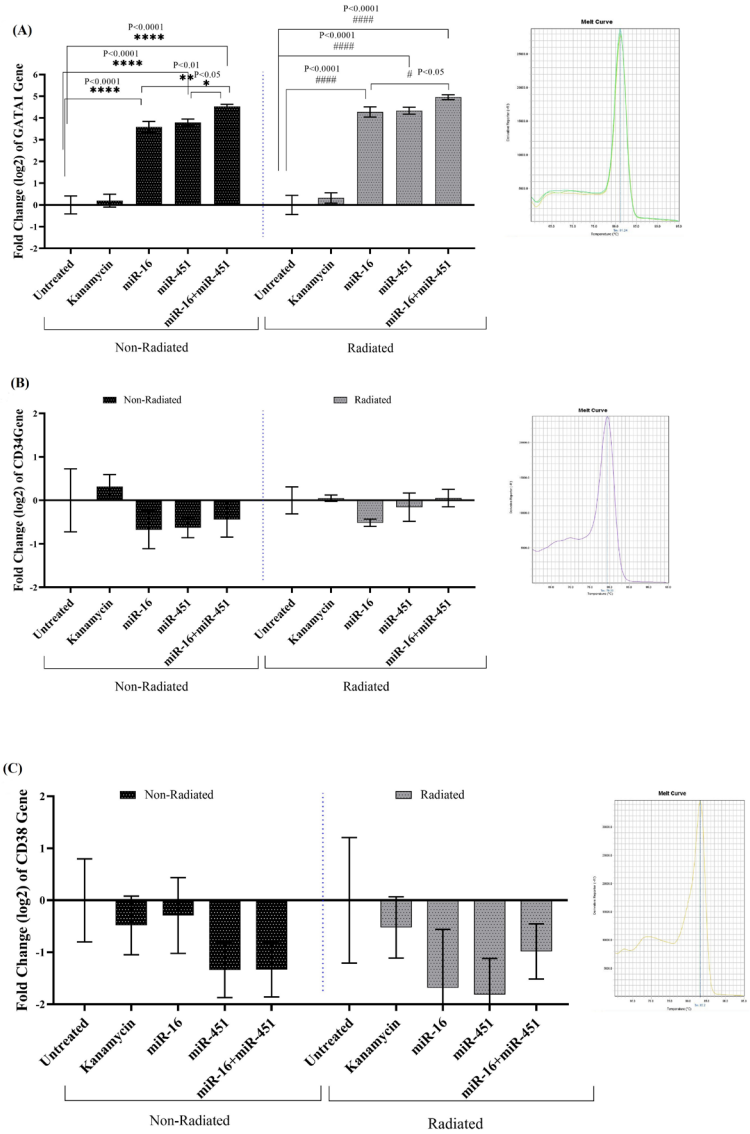


Fig. 7. The expressions of *GATA1* (A), *CD*^{34} (B) and *CD*^{38} (C) genes (Mean ± SEM) in non-radiated, and ELF-EMF radiated fibroblast cells transfused with miR-16, miR-451 and miR-16+ miR-451. The melting curves obtained by RT-PCR for each gene are showed in right. The transgenic fibroblast cells were treated with 10 mT electromagnetic field 20min every day for 7 days (n=3).

451, miR-16 and miR-16+miR-451 was investigated and showed the induced-differentiation effect to the erythroid-like progenitors with the simultaneous presence of both microRNA (miR-451, miR-16 and miR-16+miR-451) and ELF-EMF. Since the deletion of miR-451 was linked to the impairment of erythroid production in mice, one of the action mechanisms of miR-451 in enhancing the differentiation of cells into erythroid lines is the Myc inhibition.[27,28] Therefore, the overexpression of miR-451 in transgenic fibroblast cells, which led to the improvement of cell differentiation into erythroid-like lineages, could be caused by the inhibition of Myc transcription factor. Also, it was found that miR-451 is activated by *GATA-1* and its overexpression led to improved erythropoiesis in zebrafish.[29] The improvement in the differentiation of fibroblast cells into erythroid lineages in the current research may be explained by the finding that GATA-1, an essential hematopoietic transcription factor, was dramatically elevated in transgenic fibroblast cells harboring miR-451. Another study showed that the upregulation of miR-451 and downregulation of miR-150 have a positive effect on the expressions of *GATA-1*, *FOG-1*, *EKLF*, CD^{71} and CD^{235a} genes and induction of hemoglobinization.[30] However, miR-150 downregulation had no effect on erythropoiesis compared to what was observed in the control group. Therefore, they stated that the change in the expression levels of miR-451 and miR-150 can be a suitable substitute for stimulating cytokines in CD^{133+} erythroid differentiation.[30] The results of the current study indicated the role of miR-451 in erythroid-like differentiation, and the expression of two indicators, CD^{71} and CD^{235a}.

The irradiation of human fibroblast cells with 10 and 15 mT electromagnetic field increased the expressions of CD^{34} and CD^{38} genes.[31] *GATA-1* gene expression in 10 mT and 15 mT groups was not significantly different from the control group. Electromagnetic waves significantly increased the expression of CD^{34} marker on the surface of reprogrammed cells.[31] The result of the present study showed the decreased expression of CD^{34} and CD^{38} in cells transfected with miR-16 and miR-451.

Transcription factors that control the expression of genes unique to a certain lineage control the formation of mature blood cells from hematopoietic stem cells. DNA-binding proteins called GATA transcription factors are crucial for many biological processes, including hematopoiesis.[32] Among GATA family proteins, GATA-1, GATA-2 and GATA-3 are essential for hematopoiesis.[33] GATA-1 acts to promote the growth of red blood cells, megakaryocytes, eosinophils, and mast cells.[34] Mutations in GATA-1 are associated with acute megakaryoblastic leukemia, congenital erythroid hypoplasia and X-linked anemia or thrombocytopenia. GATA-1 was identified as a transcription factor associated with genes within short interactions of erythroid K562 cells.[35] The findings suggest that for chromatin connections across different cell types, H3K27ac at CTCF locations is necessary. The tissue-specific activator GATA-1 seems to be involved in erythroid cells' H3K27ac at CTCF sites.[36] Therefore, based on the role of GATA-1 in the differentiation of erythroid cells, it can be said that the increase in GATA-1 gene expression seen in transgenic fibroblast cells encoding miR-16, miR-451 and miR-16+miR-451 has played an important role in the differentiation of fibroblast cells into erythroid like progenitors.

Conclusion

In general, it can be concluded that the treatments of fibroblast cells with 10mT ELF-EMF for 20 minutes every day for 7 days, along with the upregulations of miR-16 and miR-451 are effective approaches in increasing the differentiation of these cells into erythroid like progenitors. However, to achieve large-scale *ex vivo* blood production using this approach, more studies are needed.

Acknowledgement

We would like to thank the stuff of Islamic Azad University Boushehr branch for helping us during conducting the research.

Authors' Contribution

Conceptualization: Nafiseh Karoubi, Gholamreza Khamisipour.
Formal analysis: Gholamreza Khamisipour, Narges Obeidi.
Investigation: Abbas Doosti.
Methodology: Nafiseh Karoubi.
Software: Nafiseh Karoubi.
Supervision: Gholamreza Khamisipour.
Visualization: Nahid Babaei.
Writing–original draft: Nafiseh Karoubi.
Writing–review editing: Nahid Babaei, Narges Obeidi, Abbas Doosti.

Competing interests

The authors declared that there were no conflicts of interest among authors.

Ethical Statement

Not applicable.

Funding

All research costs were personally funded by the authors.

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Research Highlights

What is the current knowledge?

√ The fibroblast cells are suitable for differentiate to erythroid progenitors

√ ELF-EMF can increase the differentiations of some types of stem cells

What is new here?

√ 10 mT ELF-EMF increased the differentiation of fibroblast cells to erythroid progenitors

√ Upregulation of both miR-16 and miR-451 increased the differentiation of fibroblast cells

√ The expressions of genes involved in hemoglobin synthesis increased in cells treated with ELF-EMF

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