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Exploring the Effects of Magnetic Fields on miR-124, miR-195, and Their Target Gene CDK6 in Bladder Cancer Cells: Insights into miRNA-Mediated Gene Regulation

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Abstarct

Background: Extremely low-frequency magnetic field (ELF-MF) is widespread environmental factors that may influence cellular behavior through non-thermal mechanisms. Recent evidence suggests that electromagnetic exposure can modulate gene expression and microRNA (miRNA) profiles in cancer cells. The aim of this research is the 50 Hz ELF-MF effect on the expression of miR-124, miR-195, and their target gene CDK6 in the 5637 bladder cancer cell line.
Methods: 5637 bladder cancer cells were cultured under standard conditions and exposed to ELF-MF for 6 hours per day over three consecutive days. The viability of cell was evaluated by MTT method. Total RNA extracted, and the change of expression of miR-124, miR-195, and CDK6 were quantified by quantitative real-time PCR.
Results: ELF-MF exposure could significant reduced of the viability of cell . The greatest reduction observed at 4 mT. Quantitative analysis revealed significant upregulation of miR-124 and miR-195 following magnetic field exposure, while CDK6 expression was significantly downregulated. Spearman correlation results demonstrated a negative correlation between miR-124/miR-195 and CDK6 expression and a positive correlation between miR-124 and miR-195.
Conclusion: Exposure to ELF-MF modulates miRNA expression and suppresses CDK6 , suggesting that magnetic fields can inhibit cell proliferation through miRNA-mediated regulation of the cell cycle. These findings provide insight into the interaction between physical environmental factors and gene regulatory networks in cancer biology.

Keywords: Bladder cancer, Extremely low-frequency magnetic field (ELF-MF), miR-124, miR-195, CDK6

1. Introduction

The biological effects of physical environmental factors on cellular behavior has been increased attention, particularly in the context of cancer biology. Among these factors, magnetic fields have attracted considerable interest. Magnetic fields are widespread physical phenomena that exist in both natural and human-made environments. Every form of life on Earth including humans, animals, plants, and microorganisms is exposed to these fields and may be affected by them either directly or indirectly [1-4]. Unlike ionizing radiation, magnetic fields could not directly damage DNA or break chemical bonds; however, growing evidence suggests that they can influence biological systems through non-thermal mechanisms, ultimately modulating intracellular signaling pathways and gene expression profiles [5]. These observations have opened new perspectives on how physical stimuli may interact with molecular regulatory networks in cancer cells. From a biophysical standpoint, magnetic fields can affect cells by altering membrane potentials, ion fluxes, and redox homeostasis. Such changes may indirectly influence transcriptional and post-transcriptional regulatory processes [6]. It has been shown magnetic fields may affect enzymes and molecular factors involved in cardiovascular diseases [7-9].

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Several studies demonstrated that exposure to static magnetic fields could lead to measurable alterations in gene expression without inducing overt cytotoxicity. Importantly, recent investigations indicate that microRNAs represent a particularly sensitive class of regulatory molecules to magnetic field. Exposure of GC-2 cells to ELF- EMFs resulted in significant changes in the expression of multiple microRNAs, despite minimal effects on cell proliferation or viability[10]. MicroRNAs (miRNAs) have been recognized as an important class of non-coding regulatory RNAs that play a prominent role in the post-transcriptional regulation of gene expression. [11-14]. In cancers, changes in expression of miRNA have been observed in the form of either upregulation or downregulation. Some miRNAs function as tumor suppressors. In contrast, others act as oncogenic miRNAs (oncomiRs), and their overexpression is associated with malignancy [13, 15, 16].

Two well-characterized miRNAs that play negative regulatory roles in cell growth and proliferation are miR-124 and miR-195. Numerous studies have demonstrated that miR-124 is a tumor suppressor across a most of cancers, and its downregulation lead to disease progression and increased cellular proliferation [17]. On the other hand, miR-195 has also tumor suppressor miRNA, and its association with the negative regulation of genes such as CDK6 has been investigated. Reduced expression of miR-195 is linked to increased activity of proliferative pathways, whereas its upregulation can lead to inhibition of these pathways[18]. Given the role of miR-124 and miR-195 in regulating cell proliferation and suppressing CDK6 in cancers, particularly bladder cancer, this research study the effects of magnetic fields intensities of 0.2, 2 and 4 mT on the expression of these miRNAs and their relationship with CDK6 expression.

2. Materials and Methods

2.1 Cell Culture

The 5637 bladder cancer cell line was sourced from the Pasteur Institute of Iran. Cells were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin, maintained at 37 °C with 5% CO_2 in a humidified incubator. Cultures were inspected daily, and every 2–3 days cells were subcultured to ensure continuous exponential growth. Only actively proliferating cells were selected for all subsequent experiments.

2.2 Magnetic Field Generation System

A uniform magnetic field was produced using an ELF-MF system based on Helmholtz coils, designed to generate a homogeneous field at the central region. The setup consisted of two coaxial circular coils of identical dimensions, each comprising 200 turns of copper wire. The separation between the coils was 15 cm. Both coils were connected to an adjustable AC power supply, allowing the application of an alternating current at 50 Hz. By controlling the current passing through the coils, uniform magnetic fields of 0.2, 2 and 4 mT were established in the central region, ensuring that the cell culture plates were fully positioned within the homogeneous field. The generated field intensity was regularly measured and calibrated using a digital gaussmeter (Lake Shore 475 DSP, USA). Measurements were taken at multiple points within the area occupied by the plates to confirm field uniformity. To minimize potential confounding effects, the control group was exposed to identical physical conditions without activating the magnetic field.

2.3 Cell Preparation and Magnetic Field Treatment

For the 5637 bladder cancer cell line, three wells of a 6-well plate were selected. In each well, 2,500 µL of cell suspension containing approximately 3×10^6 cells was seeded. The cells were divided into two groups: control (no magnetic field) and magnetic fields treatment. The treatment group was exposed to magnetic fields for 6 hours per day over three consecutive days. The control group was kept in a switched-off solenoid and similar environmental and incubator conditions, thus controlling for the effects of residual electrical currents, heat generation, mechanical vibrations, or displacement.

2.4 Viability of Cell Measurment

Cell viability and proliferation were assessed using the MTT Cell Proliferation Kit (Roche, Mannheim, Germany). Briefly, cells were seeded into flat-bottom 96-well plates suitable for cell culture at a final volume of 100 µL per well and incubated under standard conditions (37 °C, 5% CO_2, humidified atmosphere). After an initial 24-hour incubation, cells were exposed to the MFs. After incubation, MTT solution(10 µL , final concentration 0.5 mg/mL) was added to each well. Using a microplate reader (ELISA Reader, Nanodrop, Biotek, Winsooki, VT) to measure absorbance at 570 nm.

2.5 QRT-PCR

The expression levels of miR-124 and miR-195 in 5637 bladder cancer cells were evaluated using quantitative real-time PCR. Total RNA, including small RNAs, was isolated with TRIzol reagent (Sigma-Aldrich, Germany) following the manufacturer's instructions. cDNA synthesis specific for the miRNAs was performed using a miRNA reverse transcription kit (Takara, Japan).

2.6 Statistical analyses

To statistical analyses using SPSS software version 25 (IBM, Chicago, USA) was used. Group comparisons were conducted using two independent-sample tests. Independent-samples t-test was used to examine the data and determine their significance. NPar Tests and Spearman's test were also used to examine the correlation between gene expression.

3. Results

The MTT assay results demonstrated that exposure of 5637 bladder cancer cells to extremely ELF-MF reduced viability of cell. The lowest viability was observed in the 4 mT exposure group (Fig1).

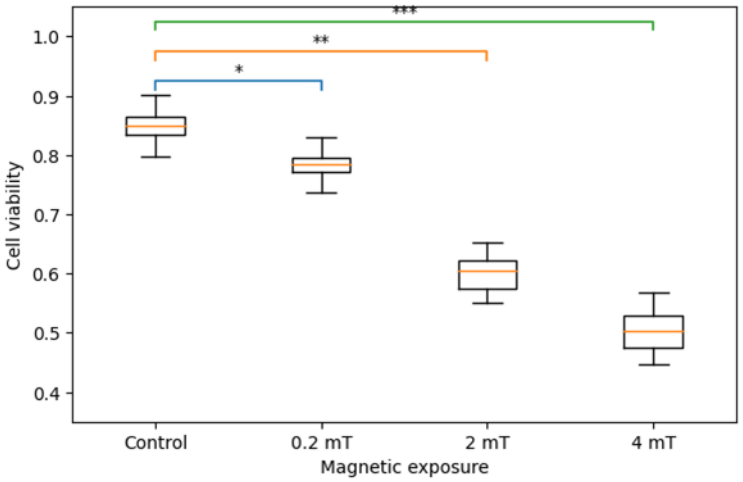

Figure 1. viability of cell after the exposure to ELF magnetic fields.

The qRT-PCR analysis indicated that ELF-MF exposure significantly lead to increase the expression level of miR-124 in 5637 cells. As illustrated in Figure 2, the fold change of miR-124 expression increased from 1.40 ± 0.15 at 0.2 mT to 1.90 ± 0.20 at 2 mT, reaching 2.60 ± 0.25 at 4 mT .

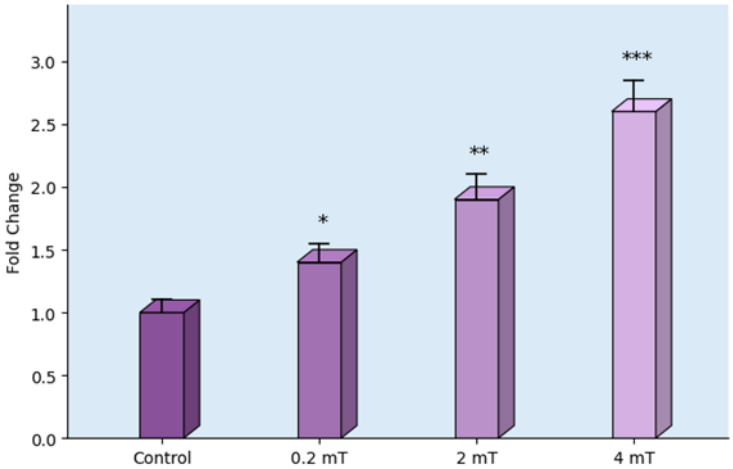

Fig 2. ofmiR - 124 expression changes after the exposure of ELF magnetic fields .

Similarly, the expression level of miR-195 was significantly upregulated following ELF-MF exposure. According to Figure 3, miR-195 expression fold change was 1.30 ± 0.12 at 0.2 mT, 1.80 ± 0.18 at 2 mT, and 2.40 ± 0.22 at 4 mT .

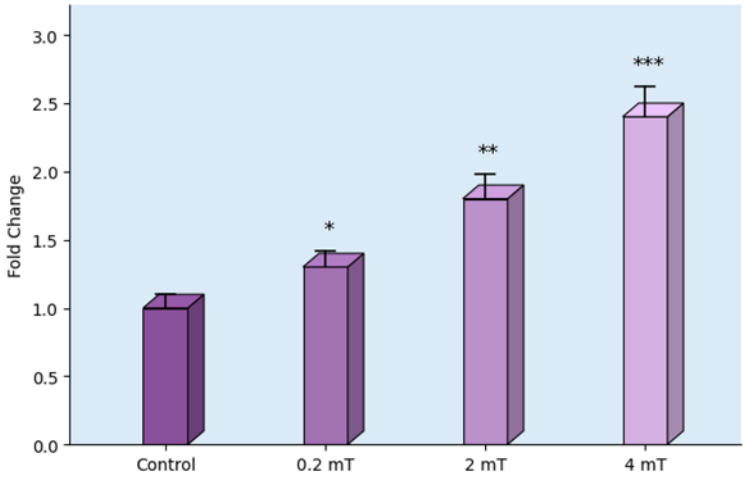

Fig 3. miR -195 expression changes after the exposure of ELF magnetic fields .

In contrast, the expression level of CDK6 was significantly downregulated after ELF-MF exposure. As presented in Figure 4, CDK6 expression decreased to 0.75 ± 0.07 at 0.2 mT, 0.50 ± 0.06 at 2 mT, and 0.30 ± 0.05 at 4 mT.

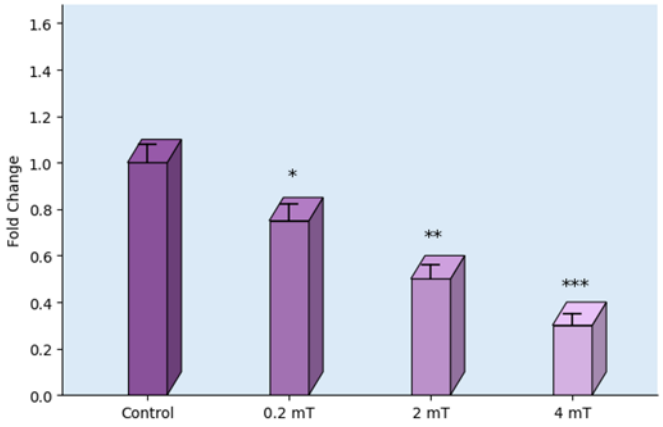

Fig 4. of DK6 expression changes after the exposure of ELF magnetic .

To evaluate the relationship between miRNAs and the target gene, Spearman correlation analysis was performed. As shown in Figure 5, a negative correlation between miR-124 and CDK6 expression levels was observed. A negative correlation between miR-195 and CDK6 was also found. In addition, a positive correlation was observed between miR-124 and miR-195 expression levels.

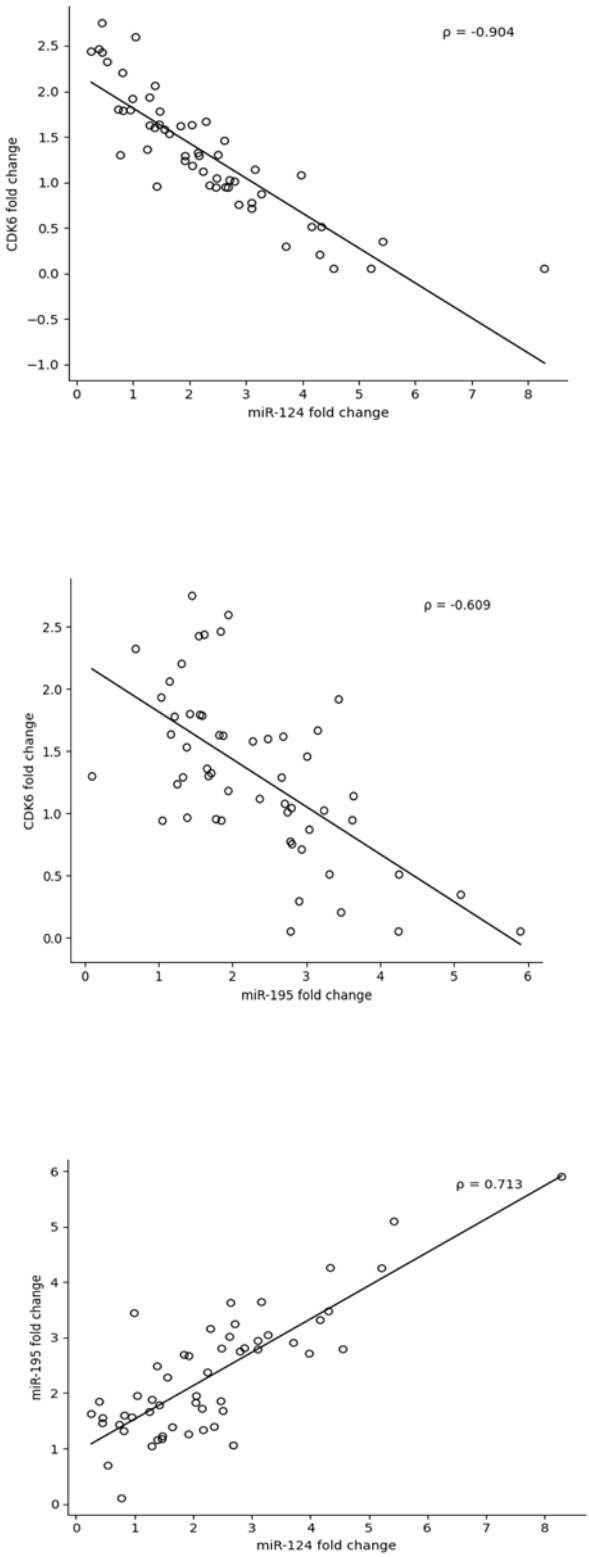

Fig 5. Bivariate correlation between CDK6, miR-124 and miR-195 expression; 54 data points were used to correlate variables by the Spearman test.

4. Discussion

ELF-MF can change cancer cell behavior by modulating key biological pathways involved in proliferation and survival [13, 19]. Although these fields are non-ionizing and cannot directly induce DNA strand breaks, they may indirectly affect tumor progression through alterations in membrane potential, calcium ion transport, oxidative stress balance, and activation of intracellular signaling cascades. [20, 21]. In this context, the present study results support the idea that ELF-MF exposure may exert anti-proliferative effects on bladder cancer cells by activating tumor-suppressive regulatory mechanisms. The observed reduction in 5637 cell viability alongside increased expression of miR-124 and miR-195 and decreased CDK6 expression suggests that ELF-MF may inhibit cell growth through modulation of miRNA-mediated control of cell cycle progression.

The data from this study indicate that exposure of 5637 bladder cancer cells to frequency ELF-MF at 0.2, 2, and 4 mT reduces cell viability and leads to a increase in the expression of miR-124 and miR-195 while decreasing CDK6 expression. In addition, miR-124 and miR-195 levels were negatively correlated with CDK6 expression, and miR-124 and miR-195 were positively correlated with each other, suggesting a coordinated regulatory response to magnetic field exposure. These findings support the idea that physical environmental factors such as ELF-MF may modulate key gene regulatory networks in cancer cells. Altering miRNA profiles contribute to proliferation, apoptosis, metastasis and invasion [22]. Both miR-124 and miR-195 have been widely characterized as tumor suppressors. miR-124 is frequently downregulated in numerus cancers, its reduced expression is related to tumor progression, whereas restoration of miR-124 expression inhibits oncogenic pathways and proliferation[17].The miR-124 tumor suppressor role has also been described in bladder cancer and other malignancies, where lower miR-124 expression correlates with increased tumorigenic[23]. MiR-195 in bladder cancer is also a tumor suppressor and other cancer types. Studies in bladder cancer cells showed that miR-195 expression reduced in tumor tissues compared with normal tissues [24]. Although the primary target of miR-195 may vary across contexts, its general role in inhibiting cell growth and promoting anti-proliferative pathways is well supported in the literature [25]. CDK6 is often overexpressed in cancers, contributing to uncontrolled proliferation. Targeted inhibition of CDK4/6 has become an effective therapeutic strategy in several cancers, underscoring the importance of this pathway in cell cycle regulation. In the present study, ELF-MF exposure reduced CDK6 expression, the negative correlation between miR-124/miR-195 and CDK6 suggests that increased expression of these miRNAs contributes to repression of proliferative signaling. Although the mechanisms by which ELF-MF influences miRNA expression are not fully understood, several possible pathways have been proposed.

Electromagnetic fields may affect intracellular signaling, ionic fluxes, and redox balance, which in turn modulate gene expression and miRNA processing. Previous reports have shown that extremely ELF could alter the expression of multiple miRNAs without inducing significant cytotoxicity, indicating that miRNA networks can be sensitive to physical stimuli [26].

The dose-response relationship observed in this study, with more pronounced effects at higher magnetic field intensities, suggests that field strength is an important determinant of the biological response. However, the broader implications of using magnetic fields as a modulator of miRNA expression in cancer therapy require further investigation.

5. Conclusion

In conclusion, our study shown that exposure to ELF-MF have a inhibitory effect on the proliferation of bladder cancer cells. This anti-proliferative effect is related to the upregulation of the tumor suppressor microRNAs miR-124 and miR-195 and the downregulation of the key cell cycle regulator CDK6. The observed negative correlations between miR-124/miR-195 and CDK6 further support the existence of a regulatory axis through which magnetic fields may influence cell cycle progression.

These findings suggest that ELF-MF can modulate gene regulatory networks at the post-transcriptional level and highlight the response of miRNA-mediated pathways to physical environmental stimuli. Although further mechanistic and in vivo studies are required, the results provide new insight into the interaction between biophysical factors and molecular signaling in cancer cells and suggest potential applications of magnetic field exposure as a complementary strategy in cancer research and therapy.

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