Effect of Exposure to 900 MHz GSM Mobile Phone Radiofrequency Radiation on Estrogen Receptor Methylation Status in Colon Cells of Male Sprague Dawley Rats

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ABSTRACT

Background: Over the past several years, the rapidly increasing use of mobile phones has raised global concerns about the biological effects of exposure to radiofrequency (RF) radiation. Numerous studies have shown that exposure to electromagnetic fields (EMFs) can be associated with effects on the nervous, endocrine, immune, cardiovascular, hematopoietic and ocular systems. In spite of genetic diversity, the onset and progression of cancer can be controlled by epigenetic mechanisms such as gene promoter methylation. There are extensive studies on the epigenetic changes of the tumor suppressor genes as well as the identification of methylation biomarkers in colorectal cancer. Some studies have revealed that genetic changes can be induced by exposure to RF radiation. However, whether or not RF radiation is capable of inducing epigenetic alteration has not been clarified yet. To date, no study has been conducted on the effect of radiation on epigenetic alterations in colorectal cancer (CRC). Several studies have also shown that methylation of estrogen receptor α (ERα), MYOD, MGMT, SFRP2 and P16 play an important role in CRC. It can be hypothesized that RF exposure can be a reason for the high incidence of CRC in Iran. This study aimed to investigate whether epigenetic pattern of ERα is susceptible to RF radiation and if RF radiation can induce radioadaptive response as epigenetic changes after receiving the challenge dose (γ-ray).

Material and Method: 40 male Sprague-Dawley rats were divided into 4 equal groups (Group I: exposure to RF radiation of a GSM cell phone for 4 hours and sacrificed after 24 hours; Group II: RF exposure for 4 hours, exposure to Co-60 gamma radiation (3 Gy) after 24 hours and sacrificed after 72 hrs; Group III: only 3Gy gamma radiation; Group 4: control group). DNA from colon tissues was extracted to evaluate the methylation status by methylation specific PCR.

Results: Our finding showed that exposure to GSM cell phone RF radiation was capable of altering the pattern of ERα gene methylation compared to that of non-exposed controls. Furthermore, no adaptive response phenomenon was induced in the pattern of ERα gene methylation after exposure to the challenging dose of Co-60 γ-rays.

Conclusion: It can be concluded that exposure to RF radiation emitted by GSM mobile phones can lead to epigenetic detrimental changes in ERα promoter methylation pattern.

Keywords
Radiofrequency (RF), DNA Methylation, Colon Cancer, Mobile Phone, Microwave
Introduction

Over the past few years, the increasing use of mobile phones has led to a rise in the general community concerns about the possible risks of its use. In addition, the global system for mobile communications has provoked the researchers’ attention on the biological effects of microwave radiation. Many studies have shown that health hazards can be triggered by cell phones [1-3].

The adaptive response is an important effect of low dose radiation. Several factors including DNA repair, cell cycle regulation, antioxidant defense and the suppression of p53 accumulation may be involved in regulating the radiation response [4, 5].

Several studies have revealed that electromagnetic fields can lead to side effects in the nervous, endocrine, immune, cardiovascular, hematopoietic and ocular systems. Despite the increasing number of reports on the effects of electromagnetic radiation (EMR) in various biological systems, no satisfactory mechanism has been proposed to explain the effects of these exposures [2, 6-8].

Recently, the International Agency for Research on Cancer reported that RF exposure is a possible carcinogen. Furthermore, there are reports indicating higher risks of cancer in heavy mobile phone users [9]. In addition, a cohort study in Denmark showed some evidence of an increased risk of skin cancer among the users of mobile phones [10].

However, some recent studies have not shown an association between the risk of early childhood cancers and mother’s exposure to mobile phone or living in the vicinity of base stations during pregnancy [4]. Moreover, this correlation was not detected in cancerous adults [3, 11].

In addition to genetic alteration, the onset and progression of cancer can be controlled by epigenetic mechanism such as gene promoter methylation. Epigenetic alterations are heritable changes in the structure and function of the genome that occur without changes in DNA sequence. Epigenetic regulation has also been established for developing new approaches to cancer therapy [12].

Recently, many studies have reported the importance of DNA methylation as a biomarker for the early detection of cancer and a tool for monitoring patients with different types of cancer. In this regard, there are extensive studies on the biological significance of tumor suppressor genes as well as the identification of methylation biomarkers in colorectal cancer [12].

Studies have also shown that methylation of ERα, MYOD, MGMT, SFRP2, P16, APC, DCC, MINT, COX2, HLFT, SOCS1, and hMLH1 gene promoters play an important role in colorectal carcinogenesis [12, 13].

Estrogen receptor alpha (ER-α), known as NR3A1 (nuclear receptor subfamily 3, group A, member 1), is encoded by the gene ESR1 (Estrogen Receptor 1), acts as ligand-activated transcription factors, and modulates gene expression by interactions with promoter response elements or other transcription factors via hormone binding, DNA binding and activation of transcription domains [14].

In spite of the fact that estrogen and its receptors are essential for sexual development and reproductive function, estrogen is involved in pathological processes including breast cancer, endometrial cancer and osteoporosis [15, 16]. ER promoter methylation seems to play a role in the early stages of carcinogenesis in several tumor sites including lymphoma, esophageal cancer and CRC [17].

Some studies have reported that CpG island methylation of the estrogen receptor (ER) is increased with age in non-neoplastic colorectal epithelium, and the same methylation occurred in most sporadic colorectal neoplasia [17]. In addition, the methylation level of the ER gene in UC patients with neoplasia was significantly higher than that in UC patients without neoplasia throughout the colorectum [18]. In addition to genetic alterations, the epigenetic modifications may be involved in
causing disruption of diseases such as autism [19] and cancer via an epigenomic side-effect of exposure to electromagnetic radiation.

On the other hand, numerous studies demonstrated that genetic changes are produced by radiofrequency (RF) radiation; however, the biological effects of RF radiation on the epigenetic factors are poorly understood. Several genes and environmental factors are involved in cancer, and electromagnetic fields may be one of these environmental factors. In this regard, colon cancer is a great model system for investigating the epigenetic mechanism of aberrant gene expression alteration [12]. Furthermore, the incidence of CRC increases due to changes in the lifestyle in Iran, and RF exposure could be a reason.

Over the past several years, our laboratories have expanded their focus on studying the health effects of exposure to some common and/or occupational sources of electromagnetic fields (EMFs) such as cellular phones [20-27], mobile base stations [28], mobile phone jammers [29], laptop computers [30], radars [21], dentistry cavitrons [31] and MRI [32, 33]. To the best of our knowledge, there is no study on the effect of RF radiation on epigenetic alteration in CRC. Therefore, the present study is an attempt to investigate whether epigenetic pattern of ERα is sensitive to RF radiation and may be adapted to epigenetic changes after the challenge dose (γ-ray).

Material and Methods

In this study, male Sprague-Dawley rats (3 weeks old weighing 200-250g) were used. The animals were purchased from the Experimental and Comparative Medicine Center at Shiraz University. Forty rats were randomly divided into 4 groups and kept in an animal care facility; food and water were supplied ad libitum. The animals in group I were exposed to cell phone radiation for 4 hours, (the rats were placed on a circle with a radius of 20 cm) and then sacrificed after 24hrs. Animals in group II were exposed to cell phone, after 24hrs irradiated by 3Gy γ (Co-60), and sacrificed after 72hrs. In group III, 10 rats were exposed to 3Gy γ radiation and group IV was used as control without exposure to RF.

A commercial mobile phone (Nokia, N70) with an average SAR of 0.95 W/kg was used to exposures. After exposure to radiation, the rats were sacrificed; the large bowel was removed and washed. The fresh samples were immediately snap frozen and stored at -80°C until processing. Then, genomic DNAs were extracted as described previously [34] and the purity of DNA was measured with Nano-drop.

**Methylation-specific PCR (MSP)**

We determined ER promoter methylation status by chemical treatment with sodium bisulfite and subsequent MSP, as described in [34]. In brief, this technique uses bisulfite Modification to convert the unmethylated but not methylated cytosine to uracil. MSP utilizes this difference to amplify specifically either methylated or unmethylated DNA and primers. The sequences of primers used for amplification of the ER promoter are shown in Table 1.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer Sequence (5’-3’)</th>
<th>Annealing Temperature, °C</th>
<th>Product Size, bp</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esr1 M-sense</td>
<td>TGAGTGTTTTGTATTCGATTTTTC</td>
<td>50</td>
<td>M=137</td>
</tr>
<tr>
<td>Esr1 M-antisense</td>
<td>ATACTTCTACTATTCCACACTCGTT</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Esr1 U-sense</td>
<td>GTGTGTTTTGTATTTTATTAGA</td>
<td>50</td>
<td>U=129</td>
</tr>
<tr>
<td>Esr1 U-antisense</td>
<td>ATACTTCTATTATTCCACACTATT</td>
<td>50</td>
<td></td>
</tr>
</tbody>
</table>
The hot-start PCR reactions were performed in a 50µL reaction volume containing modified DNA in PCR buffer provided by Taq enzyme supplier. The reaction mixture was denatured at 95°C for 5 min, after which 1.5 U Taq polymerase was added; then, it was amplified by 40 cycles, each consisting of 30s denaturation at 95°C, 45s annealing at 57°C for ER, and 30s polymerization at 72°C, followed by a single 10-min extension at 72°C. Negative controls were performed for each PCR set. 10 µL of amplified PCR products was mixed with 5 µL gel loading dye and electrophoresed on 2.5% agarose gel containing 0.5 µg/ml gel red with TBE buffer and visualized under UV illumination. The universal methylated DNA (Zymo research) was used as positive control for methylated alleles of ER. The frequencies of the methylation status of ER promoter are summarized in Table 2.

Results
As shown in Figure 1, the presence of ER methylated allele was 10/10 (100 %) for each group; whereas, the un-methylated band was variable between groups. Although un-methylated bands were detected in 100% of the control group, they disappeared in 10 rats exposed to cell phone and 3Gy gamma radiation.

In addition, rats in group II which were exposed to cell phone and then after 24hrs irradiated with 3Gy gamma (Co-60) could not compensate for the epigenetic damage and un-methylated bands were detected in 10% of the rats. Some examples of ER methylation in different treated groups are shown in Figure 2.

Discussion
In the current study, we clarified that methylation pattern of ERα is sensitive to RF radiation which may not be adapted to epigenetic changes after the challenge dose (γ-ray).

The data indicated that the rat colon epithelium appears to behave differently as compared to the human epithelium tissue in the case of ER methylation. Normal cells are completely semi-methylated and only a subset of rats exposed to radiation showed un-methylated ER allele. This marked difference between the colonic epithelium in rats and human may

<table>
<thead>
<tr>
<th>GROUP</th>
<th>methylated allele (%)</th>
<th>un-methylated allele (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (AD)</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Group II (AD+CD)</td>
<td>100%</td>
<td>10%</td>
</tr>
<tr>
<td>Group III (CD)</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Group IV (Control)</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Group I: Rats were exposed to cell phone radiation for 4 hours, Group II: the rats were exposed to cell phone radiation, then after 24hrs irradiated with 3Gy gamma radiation. Group III: the rats were exposed 3Gyγ. Group IV: control group. M, U shows the presence of methylated or unmethylated allele.
be related either to differential effects of ER expression on cellular proliferation or to differences in carcinogenic exposures in the two species.

Recently, DNA methylation and its role in tumorigenesis have become one of the hotly debated issues in molecular oncology. More recently, total hypomethylation with specific hypermethylation at individual loci was observed in cancer. More studies suggest that DNA hypomethylation may also control the gene expression and chromosomal stability [35].

Many researchers have proposed some candidate genes which are hypermethylated in several cancers including colorectal cancer (CRC) [36].

ER alpha and MYOD, p53 the cell cycle regulatory genes, cyclin A1, UDP-glucuronosyltransferase (UGT1A1) and retinoic acid receptor (RAR) are hypermethylated in colorectal cancer. This type of change in methylation appeared in an early phase of colon carcinogenesis [35]. Treatment with either the inhibitors of histone deacetylase or demethylating agents restore the normal expression of hypermethylated cells [35]. Among these genes, the methylation status of ER promoter in the lymph nodes of stage I and II CRC patients may be a useful marker for the identification of patients at a high risk for local recurrence [37].

Nowadays, the correlation of environmental chemicals and radiation with alterations of the epigenome which potentially contribute to cancer and other diseases has been proved. Evidence indicates that exposure to different sources of electromagnetic fields (EMFs) decreases the human sperm motility and also the visual reaction time in university students and radar worker [1, 30]. However, it is not known whether non-ionizing radiation directly induces changes in the epigenome of irradiated cells to increase the risk of cancer.

There is only one study that indicated the electromagnetic fields do not have enough energy to cause DNA alterations directly; however, they are able to induce epigenetic modifications in several diseases in the nervous system such as autism [19].

Therefore, our aim in current study was to investigate the ER methylation status in the colon tissue after exposure to RF.

Based on our results, exposure to mobile radiation might be dangerous due to the decrease in the content of U-allele which causes ER expression compared to non-exposure control.

Although we considered the fact that gene hypermethylation is a hallmark for cancer, it seems that the harmful epigenetic alteration could increase in M-allele or decrease in U-allele of the target gene.

For the first time, our data showed that the effect of exposure to mobile phone radiation and 3Gy gamma radiation are the same and both of them could decrease U-allele in the treated colon tissues of rats compared to the controls (p=.000). In addition, these epigenetic changes via cell phone could not be protected by challenging gamma radiation. In this regard, there is a controversy between the effects of radiation on the epigenetic alteration. In one study, researchers showed that
of Medical Sciences (SUMS), Shiraz, Iran.

Conflict of Interest
None Declared

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