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Original Article

# Revealing the Key Proteins under Telecommunications' Tower in Brain Tissue of Rats through Proteomics Approach

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## Abstract

**Introduction:** Although there are a lot of interests in telecommunications tower approaches in view of Cancer, limited researches have studied the molecular pathways, which are enriched under ELF/EMF. The aim of this study is investigating the key proteins, affected by by Tower.

**Materials and Methods:** In this study, 30 Rats under wavelength electromagnetic waves (RF900 MHz) were randomly selected. Two-dimensional electrophorese was performed to study proteomics of Rat's brain.

**Results:** Totally, 26 differentially uttered proteins (DEPs) were categorized by cytoscape network analysis.

**Conclusion:** Some key proteins in view of cancer are regulated under the face of a non-standard (unconventional) radio frequency radiation, which can induce the complement and coagulation cascades pathway.

**Keywords**: Tower, Proteomics, protein functions, Cytoscape network

## 1. Introduction

Several researches demonstrate that ELF/EM has several negative impacts on cells[1] hormonal system [2-6] REM phase [7,8] metabolomics [9-11] pathology [12,13] single and double string break of DNA [14-19] reproduction and growth[20] as well as malignancy [21-23]. On the contrary, electromagnetic fields have noteworthy influence on central nervous system (CNS) which is the favorite of many scientists. For example, one significant research shows that exposure to 60 Hz could improve social cognition in animals [24]; in addition, cognitive performance can be down under 50 Hz [25]. Besides, ELF/EMF has impact on depression or metabolic disturbances [26]. Furthermore, the 50 Hz ELF-MFs makes an influence on oxidative stress-based nervous system [27] as well as barrier permeability [28]. It has been shown that 50/60 Hz magnetic field has the ability to modify the action potential of rats; also, it affects hippocampus and neurogenesis [29- 31]. Zecca et.all showed that long exposure to ELF/EMF rises the level of μ-opioid receptors in brain. In addition, some researches have manifested that ELF-EMF changed the anxiety among rats [32, 33].

In spite of the fact that plenty of researches reported that ELF/EMFs have negative impact on organisms, their biological effects continues to remain unclear.
In this study, the profile of proteins expression of brain was followed, which can be influenced by ELF/EMFs; furthermore, the proteins that might be changed under exposures in view of cancer by proteomics approach were also observed**.**

## 2. Materials and Methods

According to the present study, 30 adult rats (200-250 g) were selected. Animals were kept in two groups and were conserved on a light cycle (12 hours OFF/ ON) and standard temperature (23 ± 2°C). The study was down under institutional guidelines for animal care.

### 2.1. ELF/EMF Exposure System

Animals which were placed in the shuttle box under wavelength electromagnetic waves (RF900 MHz) were randomly divided into control group (the 180 m range of transmission towers) and experimental group (at risk of receiving substandard dose, 100-180 m). The test was performed for two hours for each group's.

### 2.2. Tissue Preparation

The brains of animals were extracted using standard methods [34]. Naturally, samples were frozen in liquid nitrogen and were homogenized in lysis buffer and then, 100 µl of a protease inhibitor cocktail (Roche Diagnostics) was added there. Then, the homogenates were sonicated for 90s (15%amplitude for 3 cycles of 30s each). All proteins extraction steps were performed on ice. The protein concentration was subsequently resoluted by Bradford method [35]. Thirty µg of brain's protein were burdened on a 7% stacking polyacrylamide gel and then the proteins were cut out from the SDS PAGE gel. After extensive washing with water, protein-containing bands were subjected to trypsin digestion. Several steps of peptide extraction were performed in H2O/CH3CN (1:1) solutions acidified with 0.1% TFA and finally for each sample, peptide extracts were added.

### 2.3. Protein Identification

First-dimension separation of proteins, which was based on isoelectric point (If), was performed by immobilizes Ph gradient (IPG). Second-dimension separation based on molecular mass (MW) was performed by vertical large-format SDS-PAGE. Gel images were digitally converted to grayscale images and transferred to a UNIX workstation for subsequent analysis of spot densities. The gels were also stained with Sypro Ruby for the purpose of visualizing the total protein in each gel from both samples [36, 37].
SDS-PAGE gels were scanned using scanner Densitometer GS-800 (BioRad) scanner at 600 dpi in tagged image file format (TIFF). Image Master TM 2D platinum v6.0 software was then used to extract and digitize data from graphical images of scanned gels through detecting, normalizing, matching and comparing protein spots according to their volume percent, followed by primary analysis of 2D images by Quantity One software. The obtained scanned images of SDS-PAGE gels were further analyzed by Same Spot Software. After comparing the obtained 2D images with control samples, primary protein detection was performed based on the protein bands. By using the gel electrophoresis, scanned image and gels images in various articles, and by matching the molecular weight and isoelectric ph values as well as using the site www.ebi.ac.uk/IPI, several protein spots were identified.
Proteins with 1.2-fold change and Q-value less than 0.05 were determined as differentially expressed protein (DEPs). For quantification repeat analysis, CV was used for evaluating the reproducibility. CV is defined as the ratio of the standard deviation (SD) to the mean.

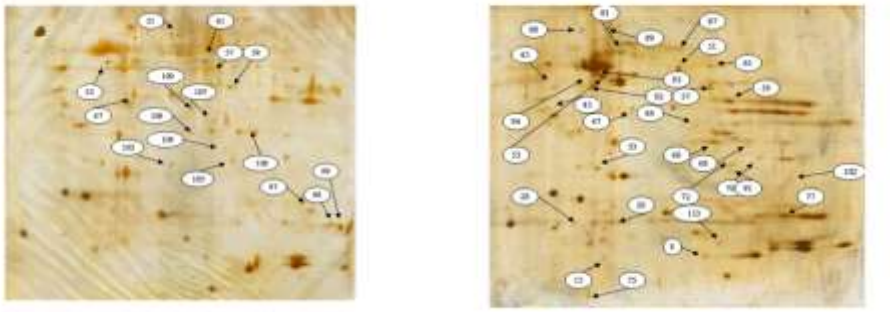
## 2.4. Network Analysis

Cytoscape network analysis database under DAVID (http://david.abcc.ncifcrf.gov/) was utilized [38].

# 3. Results

### Proteins Identification

2DE was performed in three replicates (for both Group A: 180 meters away from the telecommunication tower and Group B: at risk of receiving substandard dose, 100-180 m). In total, 46 proteins were recognized with 1% FDR (fig 1A/B).



(A) (B)

**Figure 1.** (A/B): Two-dimensional gel electrophoresis images (Group A: 180 meters away from the telecommunication tower) and experimental group (Group B: at risk of receiving substandard dose, 100-180 m) proteins were separated based on PI and MW.

DEPs for each comparisons of group/treatments analyzed. It was found that there were 26 DEPs as risk of receiving substandard dose (table 1).

**Table 1.** Details spots include: Number spots, molecular weight and isoelectric pH. (All the spots have been detected with P<0/05).

| # | Anova (p) | Fold | pI | MW | Average Normalized Volumes | |
|---|---|---|---|---|---|---|
| | | | | | Condition 1 | Condition 2 |
| **8** | 5.773e-015 | 1.3 | 5.73 | 69 | 1551.590 | 2006.000 |
| **15** | 6.994e-015 | 8.7 | 9.6 | 42 | 3103.331 | 358.000 |
| **20** | 1.549e-013 | 2.9 | 4.15 | 66 | 5.362e+004 | 1.531e+005 |
| **28** | 1.895e-013 | 2.0 | 5.72 | 82 | 3001.268 | 1517.000 |
| **33** | 2.234e-013 | 2.1 | 3.95 | 56 | 3644.273 | 7737.000 |
| **43** | 2.961e-012 | 1.6 | 3.68 | 30 | 1647.051 | 2616.000 |
| **45** | 3.359e-012 | 1.2 | 6.48 | 69 | 3241.324 | 2785.000 |
| **64** | 6.541e-012 | 5.1 | 5.36 | 82 | 4751.843 | 935.000 |
| **66** | 1.403e-011 | 6.7 | 5.29 | 80 | 4827.987 | 716.000 |
| **68** | 2.464e-011 | 2.4 | 4.01 | 67 | 3.464e+004 | 8.302e+004 |
| **70** | 2.880e-011 | 3.5 | 3.91 | 77 | 4450.108 | 1271.000 |
| **72** | 3.253e-011 | 5.7 | 4.64 | 44 | 1831.639 | 1.045e+004 |
| **75** | 3.355e-011 | 2.4 | 5.17 | 86 | 2511.354 | 1058.000 |
| **77** | 3.707e-011 | 2.4 | 5.05 | 80 | 1.633e+004 | 6719.000 |
| **81** | 4.229e-011 | 3.3 | 5.48 | 82 | 3155.665 | 959.000 |
| **87** | 7.529e-011 | 2.7 | 5.25 | 94 | 1.014e+004 | 3768.000 |
| **88** | 1.082e-010 | 2.2 | 6.07 | 51 | 887.328 | 1931.000 |
| **89** | 1.284e-010 | 3.3 | 5.08 | 16 | 5096.712 | 1.657e+004 |
| **90** | 1.331e-010 | 29.3 | 5.8 | 25 | 909.655 | 31.000 |
| **91** | 1.482e-010 | 2.5 | 5.38 | 85 | 6299.076 | 2510.000 |
| **94** | 1.672e-010 | 4.3 | 6.11 | 64 | 2018.518 | 470.000 |
| **92** | 2.316e-010 | 3.9 | 6.94 | 82 | 5458.039 | 1406.000 |
| **93** | 3.638e-010 | 1.9 | 6.8 | 26 | 3396.141 | 1802.000 |
| **96** | 4.047e-010 | 1.8 | 4.54 | 53 | 3223.657 | 5842.000 |
| **102** | 8.143e-010 | 3.4 | 5.18 | 80 | 1.226e+004 | 3556.000 |
| **113** | 8.977e-010 | 7.9 | 6.85 | 25 | 2024.858 | 255.000 |

**Network Analysis of DEPs as Risk of Receiving Substandard Dose**

26 DEPs were analyzed as risk of receiving substandard dose. Cytoscape network study signified the most vital proteins found on 26 nodes, 87 edges plus 1.36 as average node degree, and then 0.105 as avg. clustering number (figure 2).

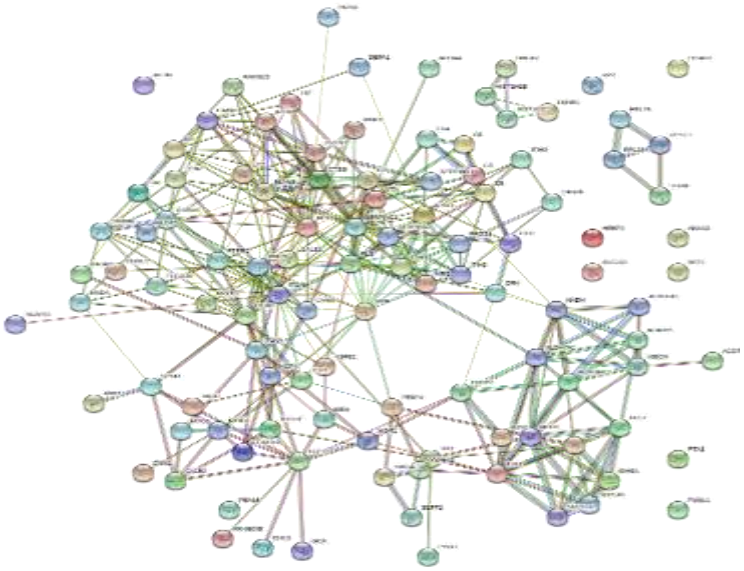


**Figure 2.** Cytoscape network analysis based on degree by Cytoscape.

A network of 26 DEPs was found as risk of receiving substandard dose (figure 2). Among all, key proteins under risk of receiving substandard dose were discovered (figure 3).

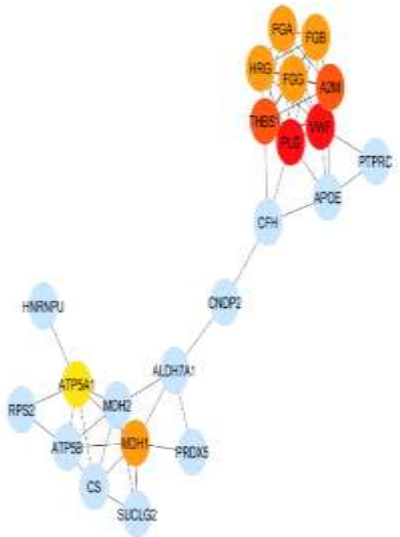



**Figure 3.** The deregulated densest protein-protein network (blue nodes: proteins, red nodes: Proteins involved in crucial pathways) in 26 DEPs.

## 4. Discussion

Although researchers have shown great interest in telecommunications tower approaches in view of cancer, limited researches have examined the biological and molecular basis of ELF/EMF [39].

In the location of radiation impacts, this study pointed at defining the molecular answer, in terms of DEPs and pathways, conferring to the dissimilar places from

Tower. Although this form of search needs to be comprehensive to frequent samples, the current work should be considered as a pilot study. These findings support the concept that presumably, the high-risk non-ionizing radiofrequency (100 to 180 m range from the mast) can induce cell-proliferation in the pathway, and hence lead to cancer.

It has long been shown that BCL-2 and BCL-XL inhibits the release of cytochrome C and thereby hinders the cell suicide/apoptosis response [40, 41]. Studies show that rats that are exposed to microwave radiation 2450 MHz for 45 minutes manifest learning loss [42]. Another study reported that Rapid Eye Movement (REM) sleep in humans decreased upon radiofrequency exposure. The electroencephalogram (EEG) during REM sleep was also altered. REM sleep is essential for learning and memory in the brain. The REM sleep is required to select and classify new observations and information at the time of awakening and reconnect them with old events [43]. In addition, a value research shows that changes in the expression levels of binding proteins and changes in calcium metabolism cause adverse changes in the hippocampal brain, attention and brain learning systems [44]. Moreover, a study showed that the activity levels might be correlated to energy production within brain mitochondria by ELF/EMF [45, 46]. In addition, melatonin as a hormone that regulates sleep-wake cycle during day and night showed variable expression levels under the influence of radio waves [47].

In this study, the proteome pattern in 100 - 180 m and further from the rig Base Transceiver Stations (BTSes) were investigated. This study has pointed to significant changes in proteins expression stages after radiation in two location of radiation (180 meters away from the telecommunication tower and at risk of receiving substandard dose, 100-180 m). Our proteomics approach, using two dimensional gel electrophoresis is an essential first stage for a biological research to define the common molecular structures related with telecommunications tower. As previously described, samples were divided into two groups according to their irradiation. Then DEPs and key pathways related to different doses were compared.

This comparison detected 26 DEPs as protein-dependent doses (table 1). The identified DEPs extracted from 2DE were enriched based on cytoscape network analysis. Cytoscape network examination signified the most significant proteins (fig. 2). Then, all in all, 26 DEPs were categorized into 10 protein classes (fig. 3), in which the most proteins associated to different places of irradiation were PLG, VWF and A2M (fig.2).

The Plasminogen, which is created by PLG, plays a vital character in pathological, wound healing, immune answer, angiogenesis, invasion then metastasis. The results manifest that the PLG is meaningfully regulated at risk of receiving substandard dose (100-180 m) with no doubt over the fact that it is extremely enriched in complement and coagulation cascades pathway.

In addition, Von Willebrand Factor Protein (VWF) which is a major platelet ligand that has been widely used as a biomarker in cancer growth and metastasis and associated inflammation, is significantly regulated at 100-180 m from telecommunications tower, highly enriched in complement and coagulation cascades too.

## 5. Conclusion

As a conclusion, the present study suggest that some key proteins in view of cancer are regulated under the face of a non-standard (unconventional) radio frequency radiation. The molecular weight and isoelectric Ph, phosphorylation or glycosylation are significantly changed; furthermore, proteomics investigations showed an important pattern for regulating

the key proteins in cell-proliferation and invasion through enrichment of the complement and coagulation cascades pathway.

Finally, it can be concluded that non-ionizing radiation emitted from transmission towers, located precisely on urban areas may act as a carcinogenic factor if the standard irradiation interval is not observed.

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## Conflict of interest

The authors declare no conflict of interest.

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