Effects of 2.4 GHz radiofrequency radiation emitted from Wi-Fi equipment on microRNA expression in brain tissue

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Abstract

**Purpose:** MicroRNAs (miRNA) play a paramount role in growth, differentiation, proliferation and cell death by suppressing one or more target genes. However, their interaction with radiofrequencies is still unknown. The aim of this study was to investigate the long-term effects of radiofrequency radiation emitted from a Wireless Fidelity (Wi-Fi) system on some of the miRNA in brain tissue.

**Materials and methods:** The study was carried out on 16 Wistar Albino adult male rats by dividing them into two groups such as sham (n = 8) and exposure (n = 8). Rats in the exposure group were exposed to 2.4 GHz radiofrequency (RF) radiation for 24 hours a day for 12 months (one year). The same procedure was applied to the rats in the sham group except the Wi-Fi system was turned off. Immediately after the last exposure, rats were sacrificed and their brains were removed. miR-9-5p, miR-29a-3p, miR-106b-5p, miR-107, miR-125a-3p in brain were investigated in detail.

**Results:** The results revealed that long-term exposure of 2.4 GHz Wi-Fi radiation can alter expression of some of the miRNAs such as miR-106b-5p (adj $p^*$ = 0.010) and miR-107 (adj $p^*$ = 0.005). We observed that mir 107 expression is 3.3 times and mir-106b-5p expression is 3.65 times lower in the exposure group than in the control group. However, miR-9-5p, miR-29a-3p and miR-125a-3p levels in brain were not altered.

**Conclusion:** Long-term exposure of 2.4 GHz RF may lead to adverse effects such as neurodegenerative diseases originated from the alteration of some miRNA expression and more studies should be devoted to the effects of RF radiation on miRNA expression levels.

**Keywords:** 2.4 GHz radiofrequency, Wi-Fi, miRNA in brain, diseases, electromagnetic fields

Introduction

The use of wireless technologies such as Wireless Fidelity (Wi-Fi) communication devices have been growing tremendously over the past years. Accessing Wireless Local Area Networks (WLAN) in houses, workplaces, public areas and schools has become a routine task in our daily lives. However, rapid development of wireless technologies has steadily increased the environmental electromagnetic field (EMF) levels. Public and scientific awareness that was previously focused on the adverse health effects of EMF emitted from mobile phones has shifted to the biological hazards of wireless equipment such as Wi-Fi. Because the health effects of such equipment are still unclear, the Council of Europe recommends restrictions on the use of mobile phones and internet access in all schools across the continent to protect young children from potentially harmful radiation (Watson 2011). Therefore, understanding the relationship between electromagnetic fields and health diseases such as reproductive disorders, cancer, etc., is very important for the public especially for young children who utilize wireless internet very frequently during adolescent years. In addition, uncontrolled wireless internet usage can turn into a habit and may continue throughout our lives without us being aware of the potential harmful effects of electromagnetic fields.

The relation between radiation and carcinogenesis is a well-known process. However, the underlying mechanism which identifies the radiation-induced genetic instability is still not fully understood. Therefore, to illuminate the underlying mechanism between the radiation and carcinogenesis, more detailed studies, which include double-strand breaks, mutations, gene expression and disruption of mitochondrial processes, cell cycle arrest, and apoptotic cell death, are necessary. In addition, the underlying interaction mechanism between the radiation and microRNA (miRNA) expression, which is a new research field, should be investigated. Several studies have already indicated radiation-induced epigenetic changes including DNA methylation and miRNA expression where miRNA profiles have been shown to be associated with cancer (Jones and Baylin 2007, Tunali and Tiryakioglu 2010, Aypar et al. 2011a, 2011b).

miRNA are small and non-protein-coding RNA molecules. They play critical roles in growth, differentiation,
proliferation and cell death by suppressing one or more target genes. miRNA may be located in the introns and exons of protein-coding genes or in intergenic regions. More than 50% of miRNA are found in cancer-associated regions of the genome or in fragile sites; this suggests that miRNA have important roles in the pathogenesis of neoplasias (Tunali and Tiryakioglu 2010). Therefore, miRNA represent new stars in the gene regulation galaxy, and there is a strong interest among researchers in different fields to understand their mechanism of action and to identify their targets (Sevignani et al. 2006).

Strooper and Christen (2010) stated that the discovery of microRNAs has revealed an unexpected and spectacular additional level of fine tuning of the genome on how genes were used again and again in different combinations to generate the complexity that underlies, for instance, the brain. They also reported that since the initial studies performed in Caenorhabditis elegans, they have gone a long way to begin to understand how microRNA pathways could have an impact on health and diseases in human. Although microRNAs are abundantly expressed in the brain, relatively few are known about the multiple functions of these RNA molecules in the nervous system. Nevertheless, they already knew that microRNA pathways play major roles in the proliferation, differentiation, function and maintenance of neuronal cells. Several intriguing studies have linked microRNAs as major regulators of the neuronal phenotype, and implicated specific microRNAs in the regulation of synapse formation and plasticity. Dysfunction of microRNA pathways is also slowly emerging as a potentially important contributor to the pathogenesis of major neurodegenerative disorders such as Alzheimer’s disease and Parkinson’s disease (Strooper and Christen 2010).

Alzheimer’s disease (AD) is a neurodegenerative disorder that currently affects nearly 2% of the population in industrialized countries. The risk of AD dramatically increases in individuals usually after age 70, and it is predicted that the incidence of AD will increase by 3-fold within the next 50 years. This progressive disease is characterized by the accumulation of plaques formed of short β amyloid (Aβ) peptides (Boissoneault et al. 2009). Most of the studies investigate interaction between the radiation and miRNA have been usually focused on the effects of ultraviolet and ionizing radiation (Simone et al. 2009, Aypar et al. 2011a, b, Zhou et al. 2012). However, a study on the interaction between radiofrequencies (RF) radiation emitted from Wi-Fi and microRNAs, especially on the interaction between Wi-Fi radiation and the brain is not available yet. Therefore, the aim of this study was to investigate the effect of chronic exposure of Wi-Fi radiation, which is widely used in daily life, on some miRNA in the brain. In this study, effects of chronic exposure of 2.4 GHz radiofrequency on miR-9-5p, miR-29a-3p, miR-106b-5p, miR-107 and miR-125a-3p were observed in the brain.

**Materials and methods**

**Subjects and animal care**

Sixteen Wistar Albino adult male rats with initial average weight of 313 ± 25 g were acquired from the Medical Science Application and Research Center of Dicle University. The rats were fed with standard pelleted food (TAVAS Inc., Adana, Turkey) in a standard Plexiglas cage. Final average weight of the animals was 348 ± 28.8 g. They were separated equally into two groups such as sham exposed (n = 8), and exposure (n = 8), and kept on a 14/10 h light/dark schedule. During the study, the ambient temperature (22°C) and the relative humidity (45%) were maintained in the normal range for these animals. All animal procedures were in agreement with the Principles of Laboratory Animal Care and the rules of Scientific and Ethics Committee of Dicle University Health Research Center.

**Exposure and field measurements**

A signal generator, which emits Wi-Fi signals at the 2.4 GHz frequency band, was used to represent the exposure system. Rats in the sham and exposure groups were placed in a Plexiglas cage (55 × 32 × 20 cm). Rats were free to move with no restriction in the cage during the study. The rats in the sham and exposure groups lived in the cage under normal circumstances. Rats in the exposure group were subject to 2.4 GHz RF radiation 24 h/d for 12 months. Rats in both groups were kept 50 cm far away from the antenna of the generator (Figure 1). The same experimental conditions were applied to the rats in the sham group, except the generator was...
turn off. Electromagnetic power density and the electrical field inside the Plexiglas cage were measured by field probe EMR 300 (NARDA, Pfulingen, Germany). The cage was surrounded with electromagnetic absorber material backed by metal to isolate outdoor electromagnetic fields from the test setup during the study duration of 12 months.

**Specific absorption rate (SAR) measurement**
Wireless Local Area Networks (WLAN) signal generator with 100 mW peak (50 mW rms) power was connected to a tuned half-wavelength dipole antenna and the dipole antenna was positioned in front of a reflector plate to direct electromagnetic signals towards Plexiglas carousel. The separation between the antenna and the top of the carousel was approximately 4 λ where λ is the free space propagation wavelength at 2.45 GHz, and the reflector plate to antenna separation was approximately 0.2 λ. To generate the far field conditions, Rayleigh distance for any antenna residing in free space must satisfy the following conditions: (i) \( R > 20D^2/\lambda \), (ii) \( R >> \lambda \), (iii) \( R >> D \), where \( R \) and \( D \) represent the distance from the antenna to the rats and the maximum dimension of the antenna (i.e., diameter of the enclosing sphere), respectively. For the current set-up, it can be assumed that the rats in the carousel reside in the far-field of the antenna. In this region of radiation, the electromagnetic signals emitted from the generator are assumed to be in the form of plane waves which represent the situation in most applications of Wi-Fi or WLAN equipment. The electric field generated by the source was measured with an electric field probe in the carousel at several locations in the absence of rats. These measured values were compared to the simulated electric field values to verify the reliability of the simulation set-up. Electromagnetic simulations were performed with CST Microwave Studio with rat voxel (volumetric pixel) data. This simulation tool utilizes a technique called finite integration technique (FIT) which is very similar to well-known finite-difference time domain (FDTD) technique, but FIT employs discretization on non-orthogonal grids using integral form of Maxwell’s equations as opposed to differential forms in FDTD. CST states that charge and energy conservation are more accurately preserved in FIT which, in turn, leads to stable numerical results in time-domain. Voxel rat model which was formed using computerized tomography scans of a rat was acquired from CST, and that model was used in the test setup using computerized tomography scans of

**Discussion**

Devices such as access points or Wireless Local Area Networks (WLAN) have been indispensable in houses, workplaces,
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public areas, schools, etc. However, widespread use of these devices naturally has been increasing the environmental electromagnetic field (EMF) levels. Additionally, new wireless devices such as wireless printers, hard discs, head phones, etc., have been also the sources for increasing electromagnetic pollution. Therefore, health effects of wireless technologies need to be studied and enlightened. It is also important to note that uncontrolled wireless internet usage can turn into a habit and may continue throughout our lives without us being aware of the potential harmful effects. Usage can turn into a habit and may continue throughout our lives without us being aware of the potential harmful effects. Usage can turn into a habit and may continue throughout our lives without us being aware of the potential harmful effects. Usage can turn into a habit and may continue throughout our lives without us being aware of the potential harmful effects.

Additionally, beta level was not observed (Dasdag et al. 2012). On the other hand, nowadays some miRNA have been accepted as active benef i ts. They showed that mice with AD long-term EMF exposure reduced brain amyloid-beta (A beta) deposition through decreased aggregation of A beta with an increase in soluble A beta levels (Arendash et al. 2010). However, Soderqvist et al. (2010) proposed that transthyretin (TTR) might be involved in the findings of RF exposure benefit in mice with AD. In one of our previous studies, we also measured beta amyloid protein in rats which were exposed to long-term 900 MHz radiofrequency radiation and any alteration in amyloid beta level was not observed (Dasdag et al. 2012). On the other hand, nowadays some miRNA have been accepted as an indicator of AD. Therefore, new strategies related to the interaction between RF and miRNA is very important in term cell phone use (918 MHz; 0.25 W/kg) provided cognitive benefits. They showed that mice with AD long-term EMF exposure reduced brain amyloid-beta (A beta) deposition through decreased aggregation of A beta with an increase in soluble A beta levels (Arendash et al. 2010). However, Soderqvist et al. (2010) proposed that transthyretin (TTR) might be involved in the findings of RF exposure benefit in mice with AD. In one of our previous studies, we also measured beta amyloid protein in rats which were exposed to long-term 900 MHz radiofrequency radiation and any alteration in amyloid beta level was not observed (Dasdag et al. 2012). On the other hand, nowadays some miRNA have been accepted as an indicator of AD. Therefore, new strategies related to the interaction between RF and miRNA is very important in

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Discussion on the health effects of wireless devices naturally has been increasing the environmental electromagnetic field (EMF) levels. Additionally, new wireless devices such as wireless printers, hard discs, head phones, etc., have been also the sources for increasing electromagnetic pollution. Therefore, health effects of wireless technologies need to be studied and enlightened. It is also important to note that uncontrolled wireless internet usage can turn into a habit and may continue throughout our lives without us being aware of the potential harmful effects of electromagnetic fields. Discussion on the health effects of radio frequencies, especially on wireless technologies such as mobile phones, began at the beginning of 1990s. At the end of contradictory discussions, the International Agency for Research on Cancer (IARC) classified mobile phones as 2B (IARC 2011). Although many of the contradictory studies on the health effects of mobile phone exposure exist, adequate studies are still not available on the side-effects of RF emitted from wireless internet equipment such as Wi-Fi.

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terms of explaining molecular interactions of RF. As mentioned above, since it is not possible to find any study on the effects of RF and miRNA interactions, we investigated the effects of 2.4 GHz Wi-Fi radiation on some of the miRNA such as miR-9-5p, miR-29a-3p, miR-106b-5p, miR-107, miR-125a-3p in rat brain.

Determination of miR-9-5p, miR-29a-3p, miR-106b-5p, miR-107, miR-125a-3p expression in brain may be associated with some diseases such as acute myeloblastic leukemia, alcohol dependence, Alzheimer’s disease, autism and diabetes, which are developed depending on the alteration in transcription of genes such as BACE1, BDNF, GAB2, PSEN1, PSEN2, SIRT1, SLC1A2 and VEGFA. Nelson and Wang (2010) observed a correlation between decreased miR-107 expression and increased neuritic plaque counts and neurofibrillary tangle counts in adjacent brain tissue. However, Van den Hove et al. (2014) and Van Spronsen et al. (2013) reported miR-107 as epigenetically regulated miRNA linked to Alzheimer’s Disease and correlate with changes in neuronal development and neuronal activity. Expression profiling following induction of neuronal activity demonstrated that 31 miRNA, including miR-107, were up-regulated by homeostatic plasticity protocols (Van den Hove et al. 2014). Huang et al. (2013) defined miR-107 and miR-103 as the strongest candidates, which are frequently deregulated in cancer. He et al. (2013) stated that low-expression of microRNA-107 inhibited cell apoptosis in glioma by up-regulation of SALL4. They demonstrated that miR-107 was down-regulated in glioma tissues and up-regulation of miR-107 suppressed glioma cell growth through direct targeting of SALL4, leading to the activation of FADD/caspase-8/caspase-3/7 signaling pathway of cell apoptosis (He et al. 2013). Chen et al. (2013a) stated that miR-107 was located on chromosome 10 and was down-regulated in glioma cell lines, and they recently confirmed that miR-107 expression was reduced in glioma tissues and cell lines. They also found that miR-107 inhibited glioma cell proliferation, migration, and invasion (Chen et al. 2013a). Sharma et al. (2013) reported decreased levels of circulating and tissue miR-107 in human esophageal cancer and observed significant down-regulation of miR-107 in neoplastic and pre-neoplastic esophageal tissues. Tu et al. (2013) defined miR-107 as one of tumor suppressor miRNA in head
and neck squamous cell carcinoma and stated that miR-107 seemed to play complicated roles in regulating stemness or the epithelial-mesenchymal transition of tumor cells. Chen et al. (2013b) also demonstrated that p53-induced miR-107 suppresses brain tumor cell growth and down-regulates CDK6 and Notch-2 expression, supporting its tumor suppressor role and utility as a target for glioma therapy. In our study we observed that miR-107 expression in rat brain was 3.3 times decreased in rat brain exposed to long-term 2.4 GHz radiofrequencies radiation. Therefore, it can be stated in light of the above discussion that cancer or some neurodegenerative disease may be triggered by or associated with long-term 2.4 GHz radiofrequency radiation exposure, which reduced the miR-107 expression in this study because of miR-107 defined as tumor suppressor and neurodegenerative agent in brain or other organs. In other words, chronic exposure of 2.4 GHz RF radiation may be accepted as one of the major risk factors for brain tumors or other diseases associated with miR-107. miR-106b-5p expression is another parameter we discussed in this study. Lu et al. (2014) investigated the role of the miR-106b and miR-93 in induction of autophagy and bacterial clearance in human cell lines and the correlation between miR-106b and autophagy-related gene 16L1 (ATG16L1) expression in tissues from patients with Crohn’s Disease (CD). They reported that miR-106b and miR-93 reduced the levels of ATG16L1 and autophagy, and prevented autophagy-dependent eradication of intracellular bacteria (Lu et al. 2014). However, they found that healthy colon tissues had low levels of miR-106b with isolated small focuses of expression in intestinal epithelia (Lu et al. 2014). In contrast, approximately 80% of the colon tissues from subjects with active CD exhibited higher levels of miR-106b. Intestinal epithelia in the actively inflamed mucosae exhibited the highest levels of miR-106b. Mucosae from subjects with inactive CD displayed a mild increase in expression of miR-106b with a predominantly epithelial distribution (Lu et al. 2014). Sampath et al. (2009) stated that low levels of expression of miR-106b might offer chronic lymphocytic leukemia (CLL) cells a mechanism whereby the apoptotic potential of p73 was repressed. Their findings illustrated that the existence of regulatory mechanisms wherein critical signaling pathways were modulated by miRNA in cancer cells (Sampath et al. 2009). Therefore, they reported that chemotherapeutic drugs that activated miR-106b could potentially circumvent the resistance associated with p53 dysfunction in CLL (Sampath et al. 2009). Xu et al. (2013) reported that miR-106b could promote the proliferation and invasion of laryngeal carcinoma cells by directly targeting RUNX3, and RUNX3 knockdown could abolish this phenotype. miR-106b was reported to correlate closely with skeletal muscle insulin resistance and type 2 Diabetes (Xu et al. 2013). Zhang et al. (2013) showed that overexpression of miR-106b resulted in mitochondrial dysfunction and insulin resistance in C2C12 myotubes whereas miR-106b loss of function improved mitochondrial function and insulin sensitivity. They also stated that miR-106b regulated skeletal muscle insulin sensitivity and inhibition of miR-106b was capable of improving mitochondrial functions and insulin sensitivity (Zhang et al. 2013). As mentioned in the studies performed on miR-106b-5p, it is obvious that alteration in miR-106b-5p expression may be associated with some diseases. In our study, we observed that miR-106b-5p expression in rat brain was 3.65 times decreased when rats were exposed to long-term 2.4 GHz radiofrequencies radiation. Hence, it can be stated that some diseases may be associated with long-term 2.4 GHz radiofrequency radiation exposure, which also reduced the miR-106b-5p expression in this study because of miR-106-5p was defined as the tumor suppressor and neurodegenerative agent in brain or other organs. Therefore, 2.4 GHz RF radiation may be accepted as one of the risk factors for prognoses of some diseases associated with miR-106-5p. In summary, we observed that 2.4 GHz Wi-Fi radiation emitted from wireless internet equipment altered the expression of two of five miRNAs investigated in this study. Our results showed that miR-106b-5p and miR-107 expression were decreased by RF radiation while miR-9-5p, miR-29a-3p and miR-125a-3p expressions were not altered. We found that miR-106b-5p and miR-107 expression decreased 3.6 and 3.3 times in the exposure group, respectively. Therefore, we state that 2.4 GHz RF radiation emitted from wireless equipment may be associated with prognoses of some brain diseases because of the relation between some diseases and alteration in miR-106b-5p and miR-107 expression. However, we note that this is the first animal study to investigate the effects of radiofrequencies on miRNA expression. The results of this study may be replicated at a larger group of animals. Further investigation on the biological aspects of microRNAs dysregulation in brain may help us better understand the pathogenesis of many diseases.

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Declaration of interest

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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