

Cellular and Molecular Responses to Radio-Frequency Electromagnetic Fields

The human exposure to nonionized EMF in connection with WPT applications is examined in this paper, highlighting various biological and biomedical aspects in connection with DNA, mutation, cell, and gene.

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ABSTRACT | In recent years, people have been exposed to many kinds of electromagnetic fields (EMFs) generated by domestic electrical appliances and mobile telecommunication devices. There is increasing public concern regarding the health risks of radio-frequency (RF) radiation, particularly that produced by mobile phones. Concern regarding the potential risks of exposure to EMFs has led to many epidemiological investigations, but the effects of EMF exposure on human and other mammalian cells are still unclear. Cellular studies of the effects of RF EMFs have been conducted more often than epidemiological and animal studies. This review provides a summary of the potential cellular effects of RF fields, including those generated by cell phones and their base stations. *In vitro* studies of the effects of RF fields can mainly be classified into those examining genotoxic and nongenotoxic effects. Genotoxic effects include DNA strand breaks, micronucleus formation, mutation, and chromosomal aberration, i.e., changes involving damage to DNA. Nongenotoxic effects refer to changes in cellular functions, including cell proliferation, signal transduction, and gene expression (mRNA and protein). The results of most recent studies show no marked effects of RF exposure at the cellular and genetic levels. However, some

studies have suggested RF effects, and these results require further investigation. As the wireless power transfer technologies are gaining more popularity, it is important that the engineering community participate in the health assessment study with medical and biological research groups. Since the electromagnetic environment due to future wireless power technologies continues to increase, biologists also have to promote the research assessment utilizing advanced technologies in the life sciences. This review paper attempts to provide an insight on the cellular and molecular responses to the RF electromagnetic fields and the understanding of such biological impacts are important for wireless power technology applications.

KEYWORDS | Cellular study; genotoxic effect; nongenotoxic effect; radio-frequency (RF) electromagnetic fields (EMFs)

I. HISTORICAL BACKGROUND OF EMF BIOLOGICAL EFFECTS

Exposure to electromagnetic fields (EMFs) has increased worldwide since the 1990s. An epidemiologic study [1] first showed that exposure to low-frequency EMFs increased childhood leukemia. The rapid increase in use of mobile phones since the late 1990s has increased exposure of human brains to electromagnetic waves, with a consequent concern regarding the occurrence of brain tumors. Studies of the biological effects of nonionized electromagnetic waves show “stimulation” by low-frequency EMFs (< 100 kHz) and a “thermal effect” of high-frequency EMFs (> 100 kHz) [64]. These studies can be classified into human epidemiological, animal (*in vivo*), and cellular (*in vitro*) studies. In this review, the results of recent cellular studies of radio-frequency (RF) EMF effects are discussed.

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Table 1 Major Criteria for the Evaluation of the Influence of EMFs

Research category		Subject	Evaluation criteria
In Vitro Study	Genotoxicity studies	Cells	Chromosomal aberration, Sister chromatid exchange, Micronucleus formation, DNA strand breaks, Mutation, Transformation
	Non-genotoxicity studies	Cells	Cell proliferation, DNA synthesis, Gene expression, Signal transduction, Ion channels, Cell differentiation, Cell cycle distribution, Apoptosis, Immune system, Reactive oxygen species
In Vivo Study		Laboratory animals (rat, mouse, etc.)	Carcinogenesis (lymphoma, leukemia, skin cancer, mammary gland tumor, liver cancer), Reproduction and development (implantation rate, fetal body weight, teratogenesis), Abnormal behavior, Neuroendocrinology mainly melatonin, Immune function, Blood brain barrier
Epidemiological Study		Human	Carcinogenesis and cancer death (brain tumor, childhood and adult leukemia, breast cancer, melanoma, lymphoma), Reproductive ability, Spontaneous abortion, Alzheimer disease
Influence on Human Body		Human	Psychological and physiological influences (fatigue, headache, anxiety, Lack of sleep, Brain waves, Electrocardiogram, memory), Neuroendocrinology mainly melatonin, Immune function

II. CELLULAR RESEARCH

Studies to evaluate RF EMF effects in cells, animals, and humans have used many approaches (Table 1) [2], [3]. *In vitro* studies are usually divided into genotoxicity and nongenotoxicity studies. The examined genotoxic effects include micronucleus (MN) formation, chromosomal aberration, primary DNA damage assessed using alkaline and neutral comet assays, sister chromatid exchange, and mutation, whereas nongenotoxic studies have examined cell proliferation and cell cycle distribution, gene expression (mRNA and protein), the immune system, transcriptomics (microarray analysis), apoptosis, and reactive oxygen species (ROS). The general focus of studies on EMF has been on the relation between EMF carcinogenicity, where all studies are considered equally important irrespective of whether the results were obtained in humans, animals, or cells. However, for evaluation of effects that may occur in humans, the results are weighted as such that the epidemiological study is more significant than the experimental animal study, which in turn is more significant than the cellular study. When looking at the accuracy and reproducibility of the study, the results of cellular studies have greater accuracy and reproducibility than experimental animal studies, which in turn have greater accuracy and reproducibility than epidemiological studies. Therefore, it is most difficult to get accurate and reproducible results for the studies that tell us most about the effects of EMF on humans.

III. GENOTOXICITY STUDIES

A. Micronucleus Formation

MN formation in the mitotic phase is frequently examined in cellular genotoxicity studies of RF EMF effects. In DNA damage and chromosomal aberration that occur during cell division, a DNA fragment may separate from the nucleus and cause MN formation. MN formation very rarely occurs spontaneously. Fig. 1 shows a cell with MN formation. The cell with MN was exposed to X-rays with 5 Gy. Most studies have shown no increase in MN formation after RF exposure at a specific absorption rate (SAR) ≤ 10 W/kg [4]–[6], however, some have shown more MN formation under these conditions [7]. Increased MN formation is observed at an extremely high SAR (≥ 50 W/kg) associated with heating [8], [9].

B. Chromosomal Aberration and Sister Chromatid Exchange

Chromosomal aberration was a typical indicator for genotoxicity used in early studies because the results can be observed visually. In cultured cells, chromosomal aberration can occur spontaneously, but this is extremely infrequent. It is well known that ionizing radiation causes DNA strand breaks, resulting in chromosomal aberration. Various types of chromosomal aberration can be observed. Some are severe, such as 1) chromosomal break; 2) ring; 3) dicentric chromosome; 4) large fragment;

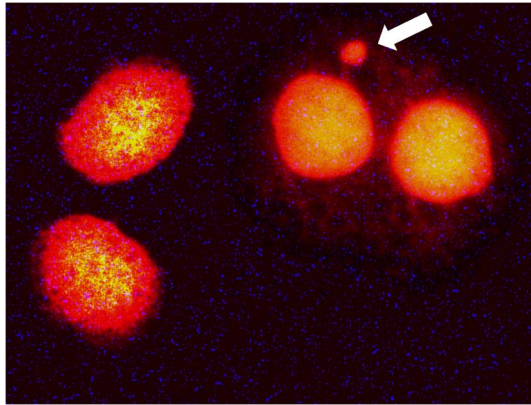


Fig. 1. A photograph of a representative MN formation. A binucleated Chinese hamster ovary K1 (CHO-K1) cell which has no MN (left), and a binucleated cell with MN (right). The arrow indicates MN (J. Miyakoshi, unpublished).

5) rearrangement; 6) loss; and 7) amplification. Other chromosomal aberrations, like gap, are slight. In a dividing cell, the chromosomes are divided into two chromatids, each consisting of one DNA molecule. Two major classes of chromatid damage are recognized: chromatid-type aberration and sister chromatid exchange (SCE). In a broad sense, chromatid-type aberrations are included as a form of chromosomal aberration. Typical chromosomal aberrations are shown in Fig. 2. An early study showed that RF exposure caused chromosomal aberration. [10], but most recent studies have shown no effect on chromosomal aberration [4], [11], [12]. Sister chromatid exchange has also not been detected in RF exposure [13], [14].

C. DNA Strand Breaks (Comet Assay)

DNA strand breaks are an index to show whether DNA strand is directly broken by cell genotoxicity. DNA strand breaks are usually examined using the so-called comet assay. Fig. 3 shows a typical photograph of comet after exposure to X-rays. DNA strand breaks after RF exposure can be detected as single-strand breaks in alkaline conditions and double-strand breaks in neutral conditions.

Several studies have shown that DNA strand breaks are increased by RF exposure [15]–[17], including with intermittent RF exposure [16], [18]. However, independently repeated experiment failed [19]. RF in combination with mitomycin C, a DNA alkylating agent, was shown to increase DNA strand breaks more than with exposure to RF alone [17]. Despite these findings, the weight of evidence supports the general consensus that RF exposure does not break DNA bonds. Scientists are appropriately conservative and will persist in supporting well-established findings until contrary evidence becomes overwhelming. Therefore, many studies have concluded that RF exposure does not cause DNA strand breaks [19]–[27].

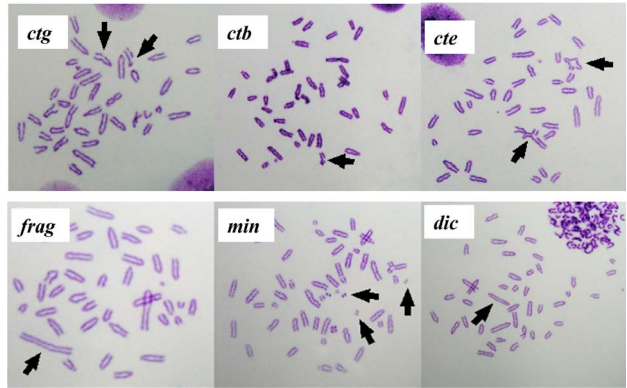


Fig. 2. Typical chromosomal and chromatid aberrations in mouse m5S. The arrows indicate the aberrations. The types of aberrations are as follows: ctg = chromatid gap; ctb = chromatid break; cte = chromatid exchange; frag = fragment; min = minute; dic = dicentric (J. Miyakoshi, unpublished).

D. Mutation

Mutation is a genotoxic effect that cannot be detected by evaluating MN formation, chromosomal aberration, and DNA strand breaks. Human cells have about 30 000 genes; therefore, it is impossible to examine for mutation in all genes. A gene is incredibly long with thousands to millions of base pairs, and it is impossible to check for alterations in this entire sequence. The idea becomes not to search for some or all specific changes. Rather, the strategy is to check the frequency of a specific mutation used as a marker. If a chemical or physical agent increases the frequency of this mutation, it probably increases the frequency of other mutations as well. Changes in bases forming the DNA strand (changes, deletion, etc.) may have great effects on cells since they can alter the functions of genes. The few studies that have examined the effects of RF exposure on mutation have all reached the conclusion that RF is unlikely to induce mutations [28], [29].

IV. NONGENOTOXICITY STUDIES

A. Cell Proliferation and Cell Cycle Distribution

Cell proliferation is a basic cellular process. In normal culture conditions, the cell proliferation rate is almost constant for each cell. However, cell proliferation is influenced by changes in the cell cycle distribution and rate of DNA synthesis. The cell cycle is divided into four phases: 1) mitotic phase (M-phase), from the beginning to the completion of a round of cell division; 2) gap 1 phase (G_1 phase), after the completion of cell division and before the beginning of DNA synthesis; 3) DNA synthesizing phase (S-phase), from the beginning to the completion of DNA synthesis; and 4) gap 2 phase (G_2 phase), after the completion of DNA synthesis and before resumption of cell division. Increased RF exposure has been shown to

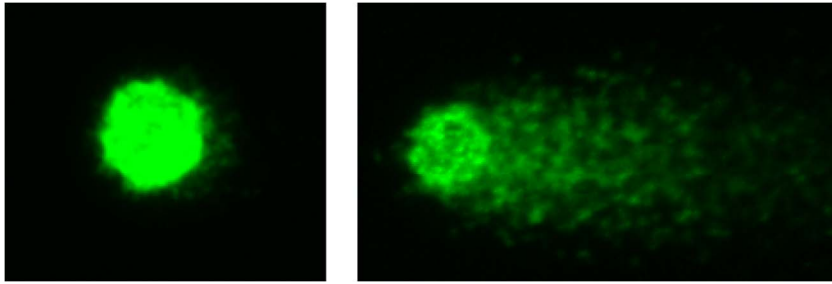


Fig. 3. Pictures of a sample result from a comet assay in Chinese hamster ovary K1 (CHO-K1) cells. After electrophoresis under alkaline conditions, the stained nuclear DNA was observed using a fluorescent microscope. Left: cell of control (no treatment). Right: cell after X-ray irradiation with 5 Gy (J. Miyakoshi, unpublished).

decrease the cell proliferation rate and influence the cell cycle distribution [30], [31], but most reports show no effect of RF exposure on cell proliferation and cell cycle distribution [32]–[34]. Furthermore, the effects of environmental factors and temperature control were not completely excluded in the results showing an effect of RF exposure, and, consequently, there is no definitive evidence of an effect of RF on cell proliferation.

B. Apoptosis

Apoptosis is a term used to describe “programmed cell death” and is understood to be a “defence mechanism” to protect cells against damage. Apoptosis is a form of cell death that is actively induced by the cell itself to maintain normal individual status. Cell death resulting from extrinsic damage and an undesirable cellular environment is referred to as necrosis, which is distinct from apoptosis. Signal transduction processes induce apoptosis in response to DNA damage induced by chemical agents and ionizing radiation, through mechanisms involving the p53 gene and caspase-3 activations. The signal transduction pathway of apoptosis is outlined in Fig. 4. Most reports of the effect of RF exposure on apoptosis are negative [35]–[37]. One report showed that increased apoptosis (caspase-3 activity) was seen as a response to serum deprivation, but no consistent effects of RF exposure were found [38]. Therefore, RF exposure is considered not to induce apoptosis.

C. Gene Expression

In simple terms, gene expression is an intracellular metabolic process in which a DNA sequence (a gene) is transcribed into mRNA and translated to protein, resulting in protein production. Effects on heat shock proteins (HSPs) have been a particular focus in studies of the effects of RF on gene expression. Production of HSPs including HSP-70 and HSP-27 is a stress response of cells to both heat and treatment with cytotoxic agents. Many groups have examined the effect of RF exposure on HSP production. At a high SAR, such as more than 20 W/kg, cell temperature and HSP production increase in RF exposure. An effect on HSP levels has also been shown using

a SAR at a level without heating, i.e., a “nonthermal effect” [39]–[42], with results showing that the increased HSP production influences signal transduction pathways being of particular interest [39], [43]. However, other studies have not found an effect of RF exposure on HSP production [44]–[48]. The exposure system, cell line, frequency, SAR, and exposure time have differed among these studies, and it is difficult to reach a definite conclusion. Gene expression is a very interesting field of research for cellular responses to RF, and further studies are required, including those on reproducibility.

The effect of RF exposure on gene expression of proto-oncogenes has also been studied, with a particular focus on c-myc, c-fos, and c-jun, which are early response genes involved in cell proliferation. The expression levels of c-jun and c-fos after RF exposure were determined using northern blot analysis [49]. The mRNA level for c-fos was unchanged, but expression of c-jun in cells exposed to RF was lower than that in the sham group. The expression of c-jun after RF exposure did not differ from sham exposure, perhaps implying recovery. These results suggest that RF exposure has a transitory inhibitory effect on c-jun expression. No significant changes in the expression levels of c-fos, c-jun, and c-myc mRNA were found by the reverse transcription polymerase chain reaction (RT-PCR) analysis after exposure of C3H 10T 1/2 cells to RF [50]. However, in frequency-modulated continuous wave (FMCW)- and code-division multiple-access (CDMA)-exposed cells, the levels of c-fos mRNA increased by about twofold and 1.4-fold, respectively. Other studies have shown no effects of RF exposure on expression of FOS, JUN, MYC, HSP27, and HSP70 [51]. At present, the results for effects of RF fields on gene expression, including HSPs and oncogenes, have been inconsistent. Many studies are ongoing, and the results of recent whole human genome studies using microarray analysis are likely to be of importance, as described in Section IV-D.

D. Transcriptomics (Microarray Analysis)

The complete sequence of the human genome has been determined, and analytical methods for screening of

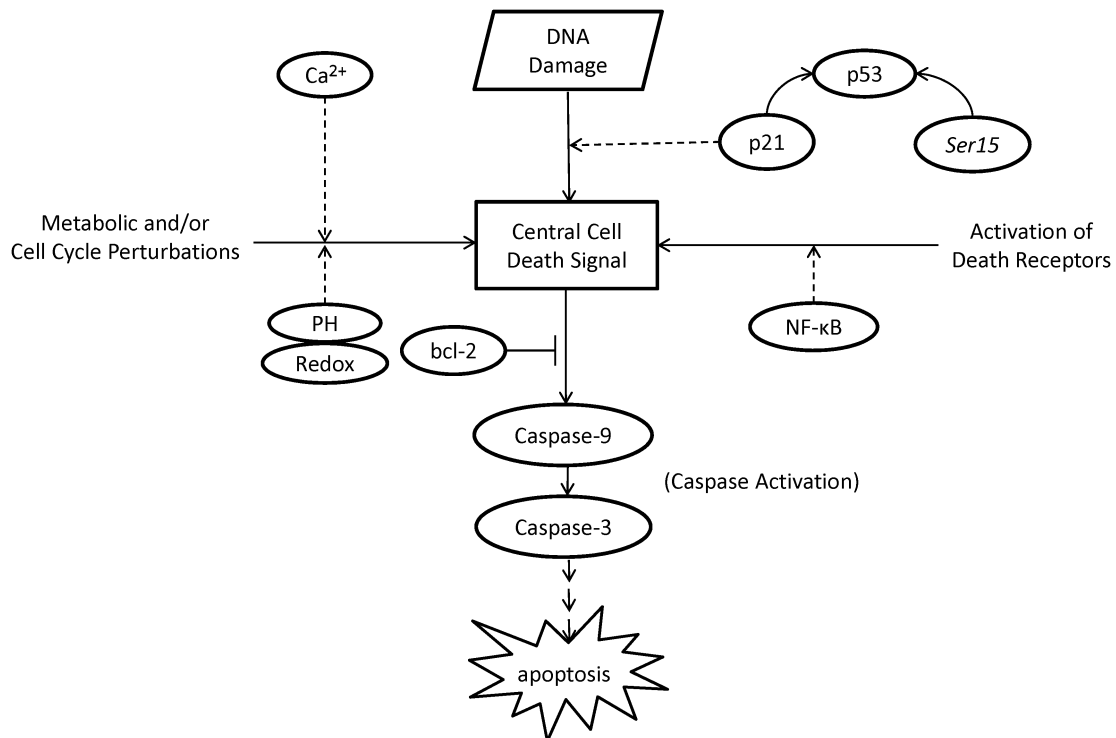


Fig. 4. An outline of the signal transduction pathway of apoptosis. The central cell death signal is activating by the metabolic and/or cell cycle perturbations, DNA damage, and/or the activation of death receptors through the action of Ca ion and/or some specific gene product of p53 or NF- κ B. In the next step, caspase activation (caspase-9 and caspase-3) occurs. Finally, cells are induced to apoptosis condition (J. Miyakoshi, unpublished).

human gene expression have been developed, including microarray analysis using DNA chips. Microarray analysis allows exhaustive assessment of the expression levels of mRNAs in a given cell. This method has been used to study RF effects. However, current microarrays do not always detect responsive genes accurately and have a high probability of detection of false positives, while small changes in expression may not be detectable. Candidate responsive genes require confirmation by RT-PCR.

Intermittent RF exposure has been shown to increase or decrease the expression of genes involved in multiple cellular functions (cytoskeleton, signal transduction pathways, metabolism, etc.) [52]. Cell-line-dependent effects of RF exposure on gene expression have also been shown [53], and cell lines with and without changes in gene expression due to RF exposure have been identified [54]. There are also several reports showing that RF exposure has no effect in microarray analysis [34], [35], [46], [55]–[57]. These reports indicate that it is difficult to find a marked cellular response to RF using microarray analysis, despite improvements in the technique.

E. Immune System

The immune system protects hosts from infection and cancer. When an external organism invades the body, immune cells attack the organism for self-protection, includ-

ing through production of many kinds of cytokines. Thus, immune cells have an important role, and effects of RF on immune cell activity have been found. Peripheral blood mononuclear cells exposed to pulse-modulated RF fields and subsequently cultured showed changes in immune activity [58] and a significantly higher response to mitogens and higher immunogenic activity (LM index) compared to control cultures [59]. In contrast, no significant effects of RF exposure and no indication that emissions from mobile phones are associated with adverse effects on the human immune system have been found based on evaluation of interleukin (IL)-1, 2, and 4 and interferon (INF)- γ and INF- α levels [60]. The levels of two proinflammatory cytokines, interleukin 6 (IL-6), and tumor necrosis factor- α (TNF- α), released into the extracellular medium after exposure of cultured astroglial and microglial brain cells to RF, did not provide evidence for an effect of RF on damage-related factors in glial cells [61]. Further studies are required to determine the effect of RF fields on the immune system.

F. Reactive Oxygen Species (ROS)

Stress due to aging, exercise, UV, and other sources increases production of reactive oxygen species (ROS). ROS include oxygen ions, free radicals, and inorganic and organic peroxides, and reactions of ROS with intracellular

DNA and lipoprotein lead to altered cellular function. Only a few studies have examined the effects of RF on ROS production. Heat and phorbol 12-myristate 13-acetate (PMA) treatment induced a significant increase in superoxide radical anions and ROS production compared to sham and/or incubator conditions [62]. No significant differences in free radical production were detected after RF exposure, and no additional effects on superoxide radical anion production were found after coexposure to RF and PMA or lipopolysaccharide (LPS). Exposure to an RF field in the presence or absence of 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a potent environmental carcinogen produced during chlorination of drinking water, did not result in induction of ROS under any of the experimental conditions investigated [63]. Thus, no study has found that ROS production is increased by RF exposure.

V. SUMMARY

For the RF exposure conditions in the cellular experiments, the frequencies used in the most aforementioned reports ranged from 800 MHz to 3 GHz. Many of them are the same signal as the RF field from cellular phones, e.g., GSM at around 900 or 1800 MHz; CDMA and time-division multiple access (TDMA) at around 800 or 1800 MHz; and the Universal Mobile Telecommunication System (UMTS) at around 900 or 1900 MHz. Some of them are continuous wave (CW) at 2450 MHz, such as the same as the RF field from a microwave oven. The SAR levels used in the most aforementioned reports range from 1.0 to 10 W/kg, except for high SAR levels at more than 20 W/kg in a few reports, and the exposure times are 30 min to 24 h.

Current understanding of the effects of RF exposure on cells can be summarized as follows. 1) RF energy does not cleave intracellular DNA directly. 2) Most genotoxicity studies have shown negative effects, except for exposure to RF fields with an extremely high SAR that results in a thermal effect. 3) Changes in gene expression associated with HSP production are an interesting cellular response to RF exposure. However, the results of studies of this effect are inconsistent, perhaps due to differences in cell lines, RF exposure system, frequency, SAR, and exposure time. Reproduction of results in different laboratories is of importance. 4) At present, microarray analysis has not provided definite evidence of an effect of RF exposure on cellular functions, including apoptosis, the immune system, and ROS production.

Generally, when we want to study the effect of one stimulus in cells, it must carefully consider the possibility of a number of artifacts. For example, in a culture environment, the selection of serum and medium, culture temperature, humidity, and CO₂ concentration must be constant in one study. For the RF exposure system in a cellular study, normal temperature control during the exposure to RF, no mechanical vibration, and, especially, the accurate measurement of SAR in cell position in the plate are re-

quired. In addition, it is conceivable that even in the same cell line, cellular response is slightly different due to differences in many cells and/or culture passages. In this review, there are some reports showing the positive effect and some other reports showing the negative effect in the study concerning one cellular criterion. Since these studies had not been done under the entirely same conditions (e.g., RF exposure system and cell type), we must be cautious to compare the results in the cellular effect. Reporting on the positive effect, especially, different researchers in different research facilities have to conduct the experiments in exactly the same conditions, to confirm whether the same result is obtained. In this way, the cellular studies in biology have a very important feature. In this review, the author hopes that the readers of the electrical engineer would understand the biological characterization techniques.

Studies on cellular RF effects are ongoing worldwide, but the published evidence regarding the effects is weak or does not allow a definite conclusion at a cellular level. The rapid development of biotechnology has increased the potential for detection of microresponses in cells and genes, and future studies of RF effects should be performed using improved biotechnological methods. Finally, RF carcinogenicity was evaluated by the International Agency For Research on Cancer (IARC) on May 23–31, 2011 [64], with RF carcinogenicity classified into Group 2B, indicating that “The agent is possibly carcinogenic to humans” [65]. At the cellular level, overall conclusion is “weak evidence” as a qualitative assessment, but not a quantitative assessment. The conclusion for each criterion was a weak evidence for the genotoxicity, such as micronucleus formation, DNA strand breaks, and chromosomal aberration; and was an insufficient evidence for mutagenicity, immune function, genes, proteins and changes in cellular signaling, and reactive oxygen species. At present, there is no clear evidence that the RF exposures at SAR levels of current ICNIRP guidelines and IEEE standards do affect the genotoxicity and/or the nongenotoxicity in cellular studies. However, further studies are required to determine the effect of RF fields on these cellular analyses.

VI. WIRELESS POWER AND HEALTH EFFECT

Electromagnetic waves in living environment are everywhere in modern society, but are not visible to the eye. The electromagnetic environment of a wide variety is impossible to avoid, and it is expected that the generation sources of electromagnetic waves will continue to increase more and more in the future society. Under such circumstances, as the application of wireless power technology evolves in our lives, some people will feel a sense of anxiety about the health effects of electromagnetic waves from the wireless power system. For example, mobile phones have become more popular since the 1990s, and the health effects of RF

waves from them, especially on the carcinogenesis of brain tumors, have become a major international issue. Medical and biological researches concerning the effect of RF waves from mobile phones have taken place in many countries to assess their risk. The World Health Organization (WHO) has launched an international project in 1996, with respect to the health effects of electromagnetic waves, which has been met with great interest. To disseminate wireless power technology in our society, we should proceed with the evaluation of the biological effects of electromagnetic waves from the wireless power system. We should study short-term and long-term safety of a person exposed to the electromagnetic waves from wireless power transmission. In such a study, cellular experiments, animal experiments, and epidemiological researches

(Table 1) are required, in order to evaluate the level of exposure, including the frequency and SAR of electromagnetic waves and exposure time.

The wireless power technology applications, such as wireless battery in cell phones, computers, as well as wireless power supply of electric vehicles, will grow exponentially in the near future. Considering the fact that the electromagnetic environment created by the future wireless power technologies continues to increase, biologists must promote their research assessment utilizing advanced technologies in life sciences. It is also important that while promoting the wireless power transfer technologies, engineering researchers participate in the health assessment study with medical and biological research groups. ■

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