Cellular Physiology and Biochemistry

Cell Physiol Biochem 2015;37:1075-1088
DOI: 10.1159/000430233
Published online: September 25, 2015
Accepted: September 02, 2015

Original Paper

8-oxoG DNA Glycosylase-1 Inhibition Sensitizes Neuro-2a Cells to Oxidative DNA Base Damage Induced by 900 MHz Radiofrequency Electromagnetic Radiation

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Key Words
Radiofrequency electromagnetic fields • DNA base damage • OGG1 • Neuro-2a cells • Reactive oxygen species

Abstract
Background/Aims: The purpose of this study was to explore the *in vitro* putative genotoxicity during exposure of Neuro-2a cells to radiofrequency electromagnetic fields (RF-EMFs) with or without silencing of 8-oxoG DNA glycosylase-1 (OGG1). ***Methods:*** Neuro-2a cells treated with or without OGG1 siRNA were exposed to 900 MHz Global System for Mobile Communication (GSM) Talk signals continuously at a specific absorption rate (SAR) of 0, 0.5, 1 or 2 W/kg for 24 h. DNA strand breakage and DNA base damage were measured by the alkaline comet assay and a modified comet assay using formamidopyrimidine DNA glycosylase (FPG), respectively. Reactive oxygen species (ROS) levels and cell viability were monitored using the non-fluorescent probe 2, 7-dichlorofluorescein diacetate (DCFH-DA) and CCK-8 assay. ***Results:*** Exposure to 900 MHz RF-EMFs with insufficient energy could induce oxidative DNA base damage in Neuro-2a cells. These increases were concomitant with similar increases in the generation of reactive oxygen species (ROS). Without OGG1 siRNA, 2 W/kg RF-EMFs induced oxidative DNA base damage in Neuro-2a cells. Interestingly, with OGG1 siRNA, RF-EMFs could cause DNA base damage in Neuro-2a cells as low as 1 W/kg. However, neither DNA strand breakage nor altered cell viability was observed. ***Conclusion:*** Even if further studies remain conducted we support the hypothesis that OGG1 is involved in the process of DNA base repair and may play a pivotal role in protecting DNA bases from RF-EMF induced oxidative damage.

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Introduction

Over the past several decades, there has been a substantial increase in mobile phone use and an expansion of the number of signal base stations located near our living areas. Thus, exposure to radiofrequency electromagnetic fields (RF-EMFs) emitted from these devices has significantly increased, provoking growing concern and debate concerning the hazardous health effects of RF-EMFs exposure. The central nervous system (CNS) is considered to be one of the primary targets of this radiation, especially with regard to carcinogenicity based on a series of accumulated data. A study [1] found that mobile phone users had an increased risk of malignant glioma and a higher rate of acoustic neuroma. Meta-analyses and adequate epidemiologic evidence suggest that there may be a causal relationship between mobile phone use and the incidence of brain tumors especially the ipsilateral brain tumor [2, 3]. In 2011, RF-EMFs have been classified as possibly carcinogenic to humans (Group 2B) by the International Agency for Research on Cancer (IARC) [4]. Recent reports about brain tumor induction among mobile phone users, especially among those who have used mobile phones daily for more than 10 years appear to agree with the notion that DNA damage in somatic cells may result in cancer induction [1, 3, 5]. However, numerous controversies remain concerning the potential of RF-EMFs to damage neural cell DNA.

Recently, numerous investigations of the effects of RF-EMFs radiation on the integrity of somatic cell genomic DNA have emerged. Previous studies observed significant increases in dicentric chromosomes (among smoker-alcoholic mobile users), sister chromatid exchanges (synergistic action with mitomycin C) [6], micro-nucleation and DNA strand breaks [7] in blood lymphocytes obtained from mobile phone users. A significant increase in erythrocyte micronuclei frequency was observed in newborn pups of mother rats maintained under 834 MHz EMF exposure at a SAR of 1.23 W/kg for 8.5 h per day throughout the gestation period [8]. Some studies [9, 10] observed a significantly high frequency of chromosomal aberrations such as dicentric and ring chromosomes and an increase in the frequency of micronuclei in EMF-exposed V79 Chinese hamster cells and human blood lymphocytes. Regarding DNA fragmentation, continuously exposed rat brain cells to microwave radiation of 2450 MHz at SARs of 0.6 W/kg (2 h) [11], and 1.2 W/kg (2 h) [12-14], they found a significant increase in DNA double- and single-strand breaks in the exposed groups. Human lens epithelial cells exposed to RF-EMFs for 2 h or 24 h exhibited single-strand breaks and double-strand breaks [15, 16], respectively, at a SAR of 3 W/kg or 4 W/kg. A recent study observed a significant increase in DNA single-strand breaks in cells of hair roots located around the human ear following exposure to RF-EMFs at frequency of 900 MHz [17]. However, these findings have not been supported by other studies [18-21]. No DNA strand breaks were detected in human blood cells after exposure to a 935 MHz basic signal at 1 or 2 W/kg for 2 h [22] or after long-term exposure to a 900 MHz Global System for Mobile Communication (GSM) signal (2 h/day, 5 days/week for 2 years) [23]. Therefore, unlike the certainty of injury effect of ionizing radiation on DNA [24], the genotoxicity of RF-EMFs exposure remains controversial, with conflicting evidence obtained under different experimental conditions.

Increasing evidence has demonstrated that exposure to RF-EMFs radiation may induce oxidative stress [25], followed by cellular oxidative damage, i.e., oxidative DNA damage [26, 27]. Oxidative stress is a condition induced by oxygen and oxygen-derived free radicals. Free radicals are commonly known as reactive oxygen species (ROS) [28], which attack DNA base and thus lead to the formation of oxidized bases, abasic (apurinic/apyrimidinic) sites, strand breaks, and DNA-protein cross-links [29]. Our previous study [30] suggested that RF-EMFs exert genotoxic effects via oxidative DNA base damage, which is mediated by ROS production, in male germ cells. Among the oxidation sites of DNA, DNA bases are regarded as extremely sensitive to ROS oxidation, especially guanine due to its low redox potential [31]. 8-oxoguanine (8-oxoG) is known to be significantly accumulated when the major DNA base guanine undergoes oxidation induced by oxidative stress *in vitro* [30] and *in vivo* [32]. 8-oxoG DNA glycosylase-1 (OGG1) is the major protective enzyme against the mutagenic effects of 8-oxoG; OGG1 functions by recognizing and removing 8-oxoG from cellular DNA

[33]. An early study [34] suggested that OGG1 plays a pivotal role in the repair of oxidative damage to nuclear DNA, thereby reducing brain damage and improving cellular function. Furthermore, a recent study [35] demonstrated that a deficiency of the base excision repair (BER) enzyme OGG1 results in enhanced ischemia-reperfusion-induced damage in mouse hearts. Inhibiting the expression of human OGG1 (hOGG1) can delay the removal of 8-oxoG, which may exacerbate oxidative DNA damage caused by cadmium and may even increase the frequency of mutations [32]. Whether OGG1 plays an important role in the formation of RF-EMFs-induced oxidative DNA lesions remains unclear.

To explore the potential for oxidative DNA lesion formation due to 900 MHz RF-EMFs exposure and the possible role of OGG1 in RF-EMFs-induced DNA damage, the present study involved a 24 h continuous exposure to 900 MHz GSM Talk signals at a SAR of 0, 0.5, 1 or 2 W/kg. The results of a formamidopyrimidine DNA glycosylase (FPG)-modified alkaline comet assay showed significant oxidative DNA base damage due to RF-EMFs exposure at 2 W/kg that was exacerbated by OGG1 RNA interference. OGG1 inhibition increased the sensitivity of Neuro-2a cells to RF-EMFs-induced DNA base damage at 1 W/kg. These data clearly suggest that OGG1 plays a vital role in the protection of DNA bases from oxidative damage induced by RF-EMFs.

Materials and Methods

Cell culture

A mouse neuroblastoma cell line (Neuro-2a) was acquired from the American Tissue Culture Collection (ATCC; Rockville, MD, USA) and maintained at 37.0 ± 0.2°C in a humidified atmosphere in the presence of 5% CO_2. Neuro-2a cells were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM; Hyclone, Logan, UT, USA) with 10% fetal bovine serum (FBS) (Hyclone) and 50 mg/ml penicillin-streptomycin (Sigma-Aldrich, St. Louis, MO, USA). Cells were dislodged for subculturing every two days. In each experiment, 2 ml of cell mixture was seeded in 35 mm plastic Petri dishes (Corning, Tewksbury, MA, USA) at 2×10^5 cells/dish for 12 h before exposure to RF-EMFs.

RF-EMFs exposure system

The exposure system that we used was provided by the Foundation for Information Technologies in Society (IT'IS Foundation, Zurich, Switzerland). The setup contained two rectangular chambers placed inside the same incubator with a common location for the air inlet so as to maintain the same environmental conditions (37°C, 5% CO_2/95% air) for the exposed and sham-exposed cells. One of the chambers was excited by an EMF signal mimicking the basic pulse structure of the GSM Talk signal at 900 MHz and the other was used for sham exposure. Cell monolayers were exposed in the H-field maximum. The larger cross-section of the chambers enabled the placement of 8 Petri dishes per field maximum. The Petri dishes were arranged in two towers, each consisting of four dishes. Tight exposure and environmental control was maintained using field sensors, temperature sensors for the air environment, and an optimized airflow system. The cooling effectivity was enhanced by two strong fans per waveguide combined with two side plates at the dish holder to channel airflow to the area of the Petri dishes. This system operated at a steady frequency of 900 MHz, and the SAR variability was below 6%, with a temperature rise of 0.02°C/(W/kg) of the average SAR value following exposure of cell monolayers and with an excellent SAR efficiency of above 50 (W/kg)/W; the temperature difference between the RF-exposed and sham-exposed chambers did not exceed 0.1°C. Blind condition was run in experiments. The computer randomly determines which of the two waveguides was exposed in each trial. The exposure conditions and monitor data were sent to the IT'IS Foundation via e-mail and decoded by that foundation following the data analysis.

Exposure procedure

At 12 h after seeding, Neuro-2a cells were subjected to 900 MHz GSM Talk signals for 24 h. To evaluate the SAR-related effects of RF-EMFs exposure, the dishes were randomly divided into the following groups: (1) 0 W/kg (negative control or positive control); (2) 0.5 W/kg (either sham exposure or RF-EMFs exposure); (3) 1 W/kg (either sham exposure or RF-EMFs exposure); (4) 2 W/kg (either sham exposure

or RF-EMFs exposure). For the positive control, cells were exposed to methyl methanesulfonate (MMS) (Sigma-Aldrich) at a concentration of 0.2 mM for 1 h before harvesting. Following exposure, the cells were simultaneously subjected to the alkaline comet assay and the FPG-modified alkaline comet assay. The same exposure procedure was performed for the measurements of ROS and cell viability. In parallel, the negative and positive controls were maintained in the same incubator. To evaluate the role of OGG1 in RF-induced DNA damage, Neuro-2a cells were divided into two groups: (1) control siRNA group; (2) OGG1 siRNA group. After the interference test, the two groups were examined via RT-PCR. To determine the effects of RF-EMFs exposure after inhibition of OGG1, the following groups were examined: (1) control siRNA group (sham exposure or RF-EMFs exposure); (2) OGG1 siRNA group (sham exposure or RF-EMFs exposure). The same exposure procedure was performed for the colorimetric cell counting kit-8 (CCK-8; Dojindo, Japan) assay, the alkaline comet assay and the FPG-modified alkaline comet assay.

RNA interference in Neuro-2a cells

Cells were transfected with OGG1-specific siRNA composed of the following sense and antisense sequences: 5'-GCUUGAUGAUGUCACUUAUTT-3' (sense) and 5'-AUAAGUGACAUCAUCAAGCTT-3' (antisense). The cells were transfected with the siRNA duplexes using Oligofectamine (Invitrogen, Carlsbad, CA, USA). OptiMEM (Invitrogen) was used as a serum-free medium to prepare working solutions of the transfection reagent and the siRNAs. Neuro-2a cells were cultured in 35 mm plastic Petri dishes at 1.5×10^5 cells/dish and transfected with siRNA targeting OGG1 (Invitrogen) or a non-targeted control siRNA (Invitrogen) using the transfection reagent LipofectamineRNAiMAX (Invitrogen) according to the manufacturer's instructions. Neuro-2a cells were transfected with siRNA for 24 h before subjected to RF-EMFs treatment, and then collected for various other treatments.

Cell viability assay

The effects of RF-EMFs exposure on cell viability were evaluated via the CCK-8 assay. According to the manufacturer's instructions, Neuro-2a cells were plated at a density of 3×10^5 cells/dish in 35 mm plastic Petri dishes and exposed to 900 MHz RF-EMFs at a SAR of 0, 0.5, 1 or 2 W/kg for 24 h; alternatively, this treatment was performed after OGG1 siRNA transfection. Then, 80 µl of CCK-8 mixture was added to the 800 µl of remaining medium per dish for 30 minutes at 37°C in the dark. Then, the medium containing CCK-8 was transferred to a 96-well plate. The absorbance at 450 nm was measured (SPECTRAFLUOR, TECAN, Sunrise, Austria), and five parallel wells were assessed in each experiment.

Assay of intracellular ROS

The intracellular ROS levels were estimated using the non-fluorescent probe 2', 7'-dichlorofluorescein diacetate (DCFH-DA) (Beyotime Company, China) according to the manufacturer's instructions with slight modifications. Cells were harvested after RF-EMFs exposure, treated with 20 µM DCFH-DA, and incubated in the dark for 30 minutes. Approximately 10^4 cells were analyzed using an Infinite™ M200 microplate reader (Tecan, Switzerland); the fluorescence wavelengths used were 488 nm for excitation and 525 nm for emission.

Alkaline comet assay and FPG-modified alkaline comet assay

An alkaline comet assay and an FPG-modified alkaline comet assay were performed to determine DNA strand breaks (single- or double-strand breaks) after exposure to the RF-EMFs system. The methods were slightly optimized compared to those previously described [30]. Briefly, cells at a density of $2.5\text{-}3 \times 10^5$ cells/ml/dish were collected after exposure to RF-EMFs or MMS and were detached via trypsinization. Then, the cells were mixed with preheated (37°C) 0.65% low melting point (LMP) agarose at a ratio of 1:10 (v/v) on Comet Slides (Trevigen, Gaithersburg, MD, USA). After pre-chilling at 4°C for 30 min, the Comet Slides were soaked in the lysis solution (Trevigen) for 2 h at 4°C. Next, the DNA on the slides was unwound for approximately 30 min in an alkaline buffer (1 mM Na_2EDTA, 300 mM NaOH, pH13) at room temperature in the dark. They were subsequently subjected to electrophoresis for 40 min, washed twice in dH_2O to remove excess reagent, and transferred to 75% ethyl alcohol for 5 min. Full-length and fragmented DNA was stained with diluted SYBR® Green I (Invitrogen) and visualized using a Leica fluorescence microscope (Germany). The FPG-modified alkaline comet assay was performed to identify DNA base damage or the levels of FPG-sensitive sites. After the lysis step, the slides were washed three times with enzyme buffer solution, and

the mixtures were treated with 60 μM enzyme buffer or FPG solution (1:1000; New England Biolabs, Ipswich, MA, USA) individually for 30 min before unwinding based on the manufacturer's instructions. The subsequent treatment procedure was performed in the same manner as the alkaline comet assay.

The levels of DNA damage were expressed as follows: (a) the tail length (TL) (measured from right border of head area to end of tail in micrometer (μm)); (b) the tail moment (TM) (calculated as the product of the tail length and the percentage of DNA in the tail in arbitrary units); and (c) the tail DNA (%) (calculated as the ratio of the DNA present in the tail to the total DNA content). The average of the scores of at least 150 nuclei from each sample in every independent experiment was used in data analysis and five independent experiments were performed. All of the comet assay parameters were assessed using a computerized image analysis system (Comet Assay Software Project, CASP Lab, Wroclaw, Lower Silesia, Poland).

Statistical analysis

Statistical analyses were performed using SPSS 13.0 software (SPSS Inc., Chicago, IL, USA). Differences among multiple groups were evaluated using a one-way ANOVA with Dunnett's post hoc test. Two-tailed Student's t-test was used to determine differences between control and experimental groups as previously described [30]. The values were expressed as the means ± standard error of the mean (SEM) of five independent experiments. Differences were considered to be statistically significant at $p < 0.05$.

Results

RF-EMFs exposure does not induce cytotoxicity or DNA strand breaks

After exposure to 900 MHz RF-EMFs radiation at a SAR of 0, 0.5, 1 or 2 W/kg for 24 h, no significant differences were found between the sham and RF-EMFs exposure groups (Fig. 1A). DNA fragmentation was quantified using CASP Lab software to analyze the tail

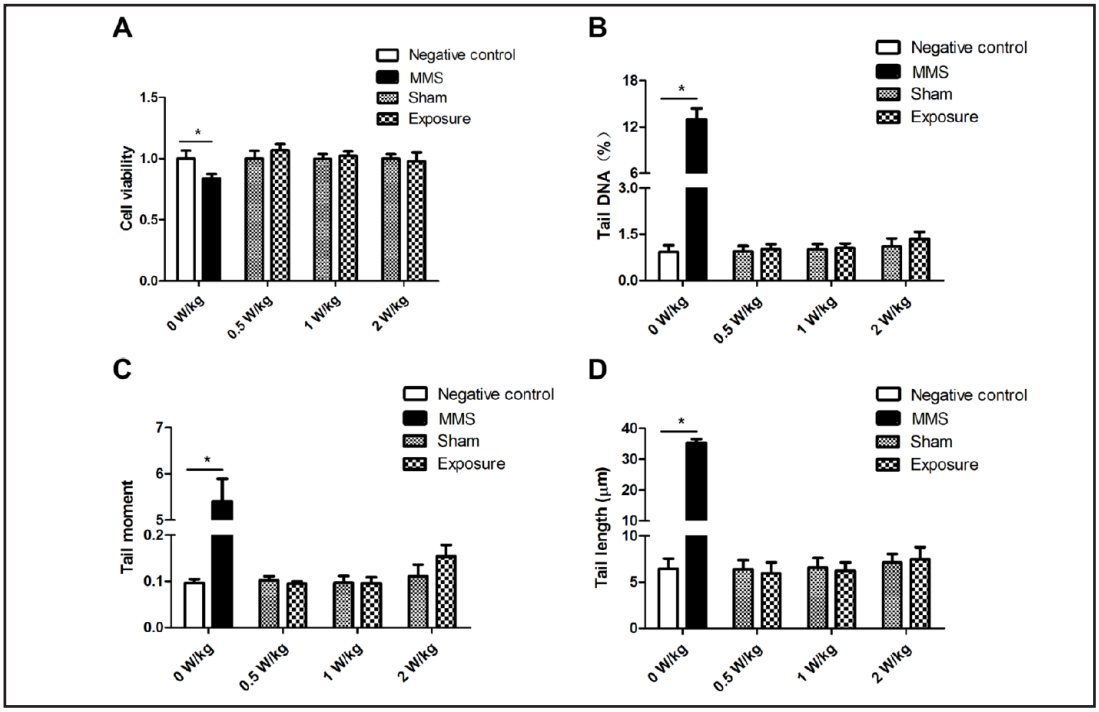

Fig. 1. The effect of RF-EMFs radiation on cell viability and DNA strand breakage in Neuro-2a cells. Cell viability was examined via a CCK-8 assay and MMS (0.3 mM) was used for positive control. The DNA migration pattern of Neuro-2a cells was evaluated via an alkaline comet assay after exposure to RF-EMFs at a SAR of 0, 0.5, 1, or 2 W/kg, or to MMS (0.2 mM) as a positive control; a negative control group was also evaluated. DNA strand breaks in all groups were measured as tail DNA (%), tail moment, and tail length. More than 150 nuclei were analyzed in each group for each experiment. The bars represent the mean ± SEM values from at least 5 separate experiments. *p<0.05 compared with the negative control group.

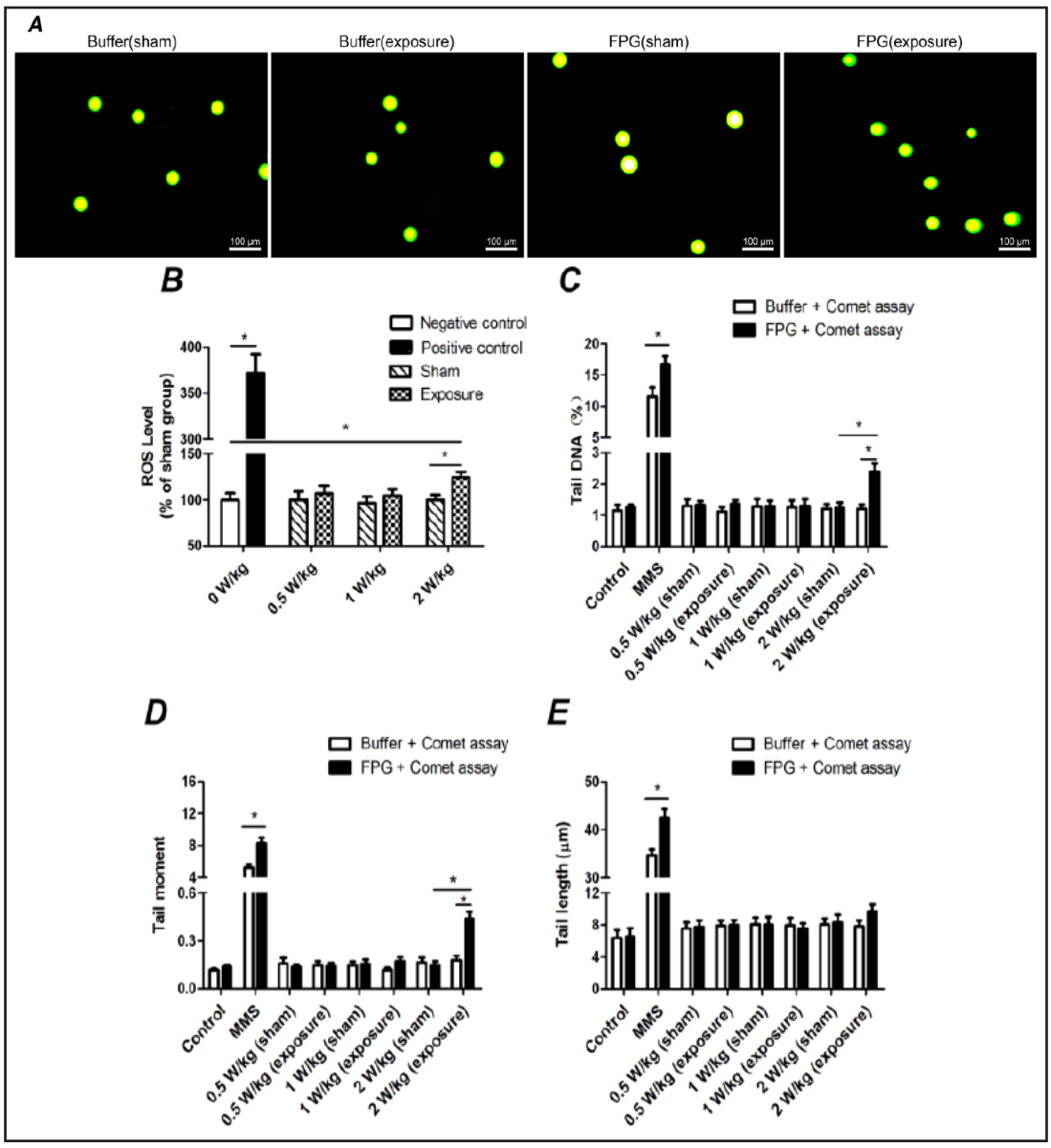

Fig. 2. The effect of RF-EMFs exposure on DNA base damage and the ROS levels in Neuro-2a cells. (A) Comet assay images of the groups subjected to sham or RF-EMFs exposure at a SAR value of 2 W/kg, as measured by a buffer-based or FPG-modified alkaline comet assay. (B) The ROS values are expressed as the increases in fluorescence intensity at a SAR of 0, 0.5, 1 or 2 W/kg. (C, D and E) Oxidative DNA base damage in Neuro-2a cells after exposure to RF-EMFs at 0.5 W/kg, 1 W/kg or 2 W/kg for 24 h or to MMS (0.2 mM) for 1 h, as measured by buffer-based or FPG-modified alkaline comet assays. The levels of DNA base damage were determined by tail DNA (%), tail moment, and tail length. More than 150 nuclei were analyzed from each group for each experiment. The bars represent the mean ± SEM values from at least five independent experiments. * $p<0.05$ compared with the FPG-treated control group and the buffer-treated RF-EMF-exposed group.

DNA (%) (Fig. 1B), tail moment (Fig. 1C) and tail length (Fig. 1D). The graphs showed that for the positive control (MMS-treated) group, all comet assay parameters were significantly increased, which suggested that the MMS dose effectively induced DNA strand breaks and that the comet assay functioned appropriately. However, no differences in DNA migration were observed after RF-EMFs exposure at a range of SARs.

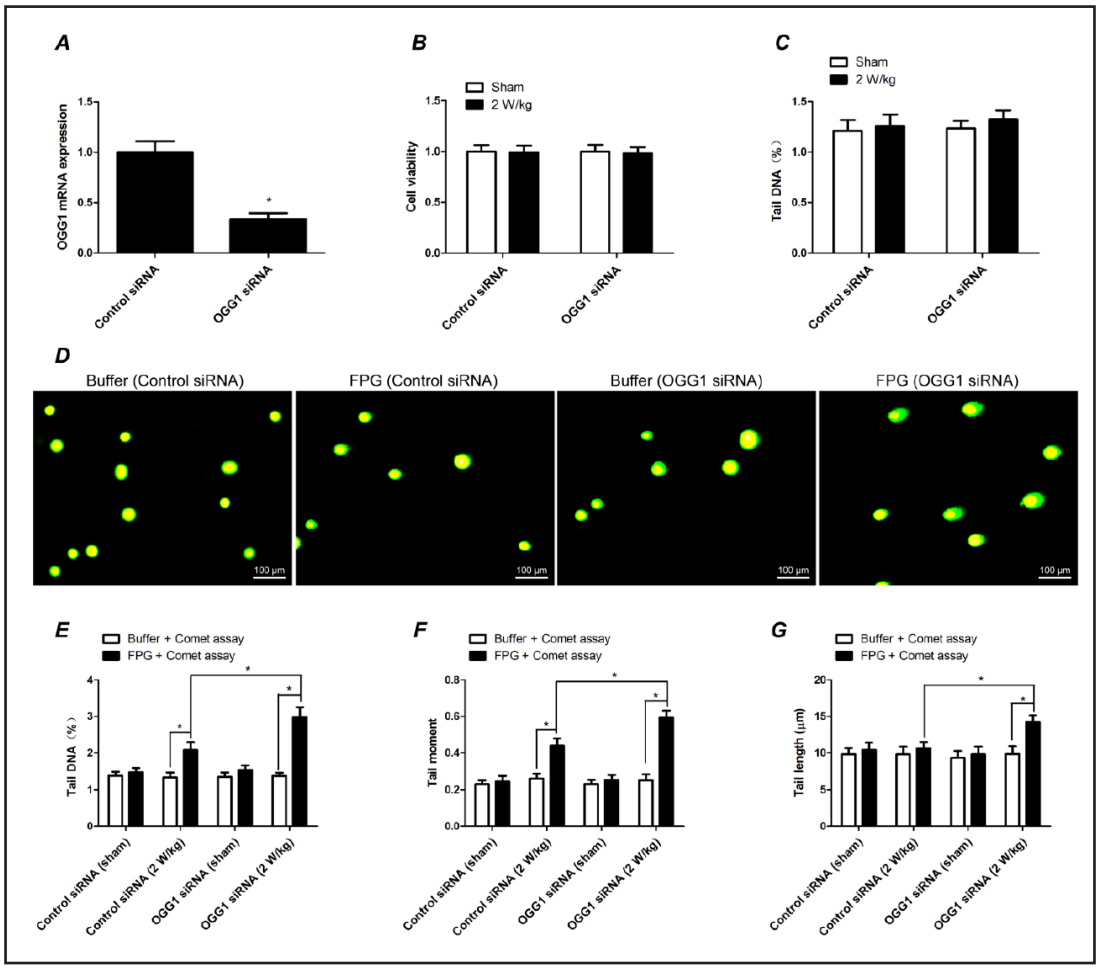

Fig. 3. OGG1 inhibition following RF-EMFs exposure at 2 W/kg in Neuro-2a cells leads to the exacerbation of DNA base damage but does not induce DNA strand breakage or changes in cell viability. (A) Cells were harvested 24 h after transfection with OGG1 siRNA or control siRNA, and the OGG1 expression level was quantified using RT- PCR. (B) The viability of Neuro-2a cells was detected via a CCK-8 assay after OGG1 knockdown followed by sham or RF-EMF exposure at 2 W/kg. (C) The level of DNA strand breakage was detected via an alkaline comet assay. (D) Images from the FPG-modified alkaline comet assay after OGG1 silencing following sham or RF exposure at 2 W/kg. (E, F and G) The levels of DNA base damage as measured by tail DNA (%), tail moment and tail length. The bars represent the mean ± SEM values from at least five independent experiments. * p <0.05.

RF-EMFs exposure at 900 MHz induces oxidative DNA base damage

An FPG-modified alkaline comet assay was performed to detect DNA base damage in Neuro-2a cells after exposure to RF-EMFs and MMS. The 2 W/kg group (Fig. 2A) exhibited a significantly increased tail DNA (%) (Fig. 2C) and tail moment (Fig. 2D), but not tail length (Fig. 2E), compared with the buffer-treated exposure group ($p < 0.05$) and the FPG-treated control group ($p < 0.05$). However, these remarkable increases were not detected in the 0.5 W/kg or the 1 W/kg RF-EMFs-exposed groups compared with the sham-exposed groups.

The intracellular ROS levels of these groups were also detected. Concomitant with the results of the FPG-modified alkaline comet assay, both the positive control group and the 2 W/kg RF-EMFs-exposed groups showed significantly increased ROS levels compared to the negative control group and the 2 W/kg sham-exposed group. The 0.5 W/kg and 1 W/kg groups showed no differences between the RF-EMFs-exposed and sham-exposed conditions (Fig. 2B).

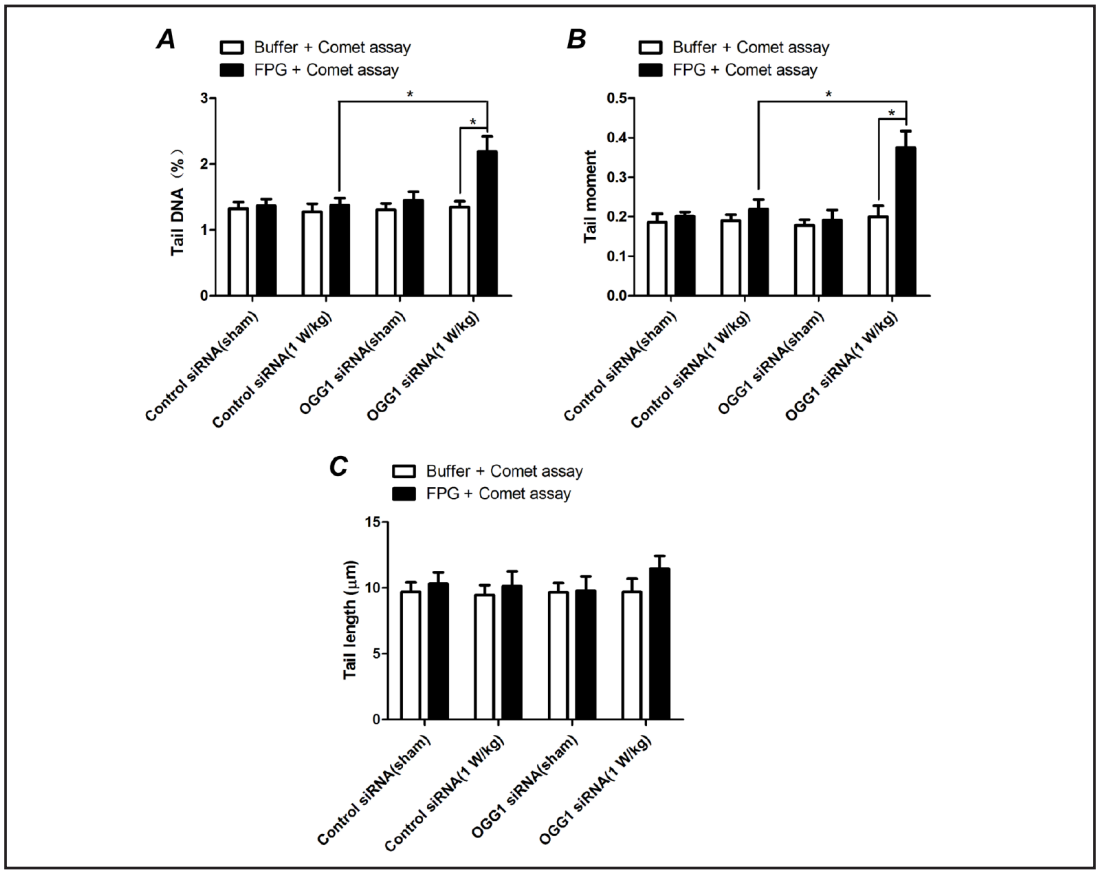

Fig. 4. OGG1 inhibition in Neuro-2a cells leads to DNA base damage following RF-EMFs at 1 W/kg exposure. Cells were transfected with OGG1 or control siRNA for 24 h, following sham or RF-EMF exposure at a SAR of 1 W/kg; then, an FPG-modified alkaline comet assay was performed. The amount of DNA base damage was measured as tail DNA (%) (A), tail moment (B), and tail length (C). The bars represent the mean ± SEM values from five independent experiments. * p <0.05.

OGG1 inhibition increases DNA base damage following RF-EMFs exposure at 2 W/kg

To investigate whether OGG1 was involved in protecting Neuro-2a cells from RF-EMFs exposure-induced DNA damage, siRNA was used to inhibit the intracellular mRNA expression of OGG1. OGG1 siRNA transfection for 24 h led to a clear decrease in OGG1 mRNA compared with control siRNA transfection (Fig. 3A). Then, the OGG1-silenced Neuro-2a cells were subjected to the same RF-EMFs exposure treatments as described above. RF-EMFs exposure at 2 W/kg did not affect the viability of Neuro-2a cells in either the OGG1 or the control siRNA-transfected group (Fig. 3B). The alkaline comet assay was used to further investigate DNA strand breakage. No significant differences were observed between the control siRNA-transfected RF-EMFs-exposed group and the OGG1 siRNA-transfected sham-exposed group. These results indicated that RF-EMFs exposure at 2 W/kg did not induce DNA strand breakage, as demonstrated by tail DNA (%), after OGG1 inhibition, as shown in Fig. 3C. Subsequently, an FPG-modified alkaline comet assay was performed to detect DNA base damage following RF-EMFs exposure at 2 W/kg (Fig. 3D). After statistical analysis, for the OGG1-silenced RF-EMFs-exposed cells at 2 W/kg, the tail DNA (%) , the tail moment and the tail length were significantly increased, compared with the corresponding cells with buffer treatment group ($p<0.05$) and sham-exposed group ($p<0.05$) (Fig. 3E, 3F and 3G).

RF-EMFs exposure at 1 W/kg induces DNA base damage after OGG1 inhibition

We investigated the possible impact of RF-EMFs exposure at 1 W/kg on DNA base damage after OGG1 inhibition. OGG1-silenced cells exposed to RF-EMFs at 1 W/kg with

FPG treatment showed significant increases in tail DNA (Fig. 4A) and tail moment (Fig. 4B), but not tail length (Fig. 4C), compared to the corresponding cells with buffer treatment. However, no apparent difference in DNA migration was detected between the control siRNA sham-exposed group and OGG1 siRNA sham-exposed group.

Discussion

In the present study, we demonstrated that RF-EMFs exposure at 0, 0.5, 1 or 2 W/kg does not lead to DNA strand breakage in the presence or absence of OGG1 inhibition. However, OGG1 inhibition increases the extent of DNA base damage induced by RF-EMFs exposure at 2 W/kg. Moreover, OGG1-silenced cells exhibited obvious DNA base damage following RF-EMFs exposure at 1 W/kg. The current data suggest that OGG1 may play an important role in protecting against RF-EMFs exposure-induced oxidative DNA base damage.

Mobile phones using the 900 MHz systems are used worldwide and these devices emit EMFs. Studies from other laboratories suggest that RF-EMFs may have adverse effects on human health including alterations in the expression pattern of cytoskeleton regulating factors, decreasing of cell viability of the rat cortical neurons, negative effects on semen quality et al. [36-38]. The GSM signal at 2 W/kg affected qualitative and quantitative features of hippocampal pyramidal cells [39]. The potential biological effects of such exposure should not be ignored. Given the long time use of mobile communications in everyday life, whether RF-EMF exposure affects human health is a major concern. However, their genotoxic effects remain controversial. The 1.6 and 2.0 W/kg safety standards were established by the USA and the International Commission on Non-Ionizing Radiation Protection (ICNIRP) [40]. Whether RF-EMF exposure affects DNA integrity at a safety standard is unknown. Thus, 0.5, 1, and 2 W/kg were selected as the doses of 900 MHz RF-EMFs radiation in this study. The experiments showed that the viability of Neuro-2a cells was not influenced in any of the groups tested; this finding was consistent with previous studies [41] indicating that the SAR of 2 W/kg have insufficient stimulus to influence the viability. Exposure to 900 MHz RF-EMFs did not induce DNA strand breaks. No evidence of DNA strand breaks was apparent in previous studies performing alkaline comet assays on mouse fibroblast cells [42], human glioblastoma cells [43], human ES1 diploid fibroblasts cells, Chinese hamster V79 cells [44], or human white blood cells [45]. However, DNA strand breaks were found in human trophoblast cells [46] and in sperm [47]. The discrepancy between these studies and our data is because RF-EMFs exposure may induce DNA damage in a manner that partially depends on the exposure duration time, the intensity of the RF-MFs, as well as the different treatments and cell types used in experiments. The present study provides evidence that 900 MHz RF-EMFs have insufficient energy to directly induce DNA strand breaks, and this finding is in agreement with previously obtained results *in vivo*[48].

Previous studies have suggested that RF-EMFs may stimulate ROS generation both *in vivo* [49] and *in vitro* [50]. Within the structure of DNA, DNA bases are considered to be highly sensitive to ROS oxidation, and guanine is particularly susceptible to ROS-induced modification because of its low redox potential [51]. Therefore, we speculated that DNA bases, particularly purines, might be more sensitive to low-energy RF-EMFs radiation than DNA strands. In this study, an FPG-modified alkaline comet assay was used to detect damage to DNA bases, especially guanine. We demonstrated that 900 MHz RF-EMFs exposure at 2 W/kg significantly induced oxidative DNA base damage, as demonstrated by the tail DNA (%) and the tail moment but not the tail length. These inconsistent results may be attributed to the fact that the tail length only represents the length of the damaged DNA, whereas the other comet assay parameters represent not only the length but also the quantity of damaged DNA [52]. In addition, the tail DNA (%) and the tail moment showed more significant discrepancies and stronger correlations than the tail length, as previously described [30]. Several authors have presented evidence of excessive free radical formation after RF-EMFs exposure [47, 53]. Moreover, RF-EMFs exposure was associated with excess production of

free radicals, especially ROS [47, 48, 53], as also concluded in the present study. The finding that the levels of DNA base damage were concomitant with similar increases in the ROS levels suggested that base damage might be resulted from oxidative stress. However, it is unknown how the ROS levels increase following RF-ENFs exposure. A study suggested that RF-EMFs exposure might elevate the levels of ROS by stimulating plasma membrane NADH oxidase or by decreasing the activity of antioxidant enzymes [54]. Therefore, further investigation is needed to elucidate the mechanisms underlying RF-EMFs radiation-induced ROS generation.

There are many studies concerning the role of several genes in radio sensitivity such as BAG-1 (bcl-2 associated athanogene), RVI (regulatory volume increase), etc [55, 56]. However, genes involved in RF-EMFs sensitivity were unclear. Oxidative DNA damage caused by free radicals and other oxidizing agents is mainly repaired by BER, which involves the excision of a modified base in the first step from DNA by a DNA glycosylase that hydrolyzes the N-glycosidic bond between the modified base and the sugar moiety, generating an apurinic/apyrimidinic (AP) site[33]. Subsequent incision by APE results in single strand breaks and a single base gap as a repair intermediate. A DNA polymerase fills in the gap with the correct nucleotide. Finally, a DNA ligase completes the repair process and restores the integrity of the helix by sealing the nick. OGG1 is a major DNA glycosylase involved in BER following oxidative damage to nuclear and mitochondrial DNA (mtDNA). As a major system for repairing oxidative DNA damage, the DNA BER pathway is initiated by the activity of lesion-specific DNA glycosylases that recognize and excise damaged DNA bases, particularly 8-oxoG, which is an oxidized form of guanine that is a major oxidative DNA base lesion implicated in carcinogenesis and aging in several biological systems [57, 58]. Some studies [59-63] have provided evidence supporting the significant effect of OGG1 on the removal of 8-oxoG, thereby protecting against DNA oxidation; such a mechanism aids in avoiding various types of oxidative DNA damage-associated disease processes such as tumorigenesis or carcinogenesis [64], inflammation [65], and neurodegeneration [66]. To investigate the role and the effect of OGG1 in RF-EMFs-induced oxidative DNA damage, cultured Neuro-2a cells were transfected with siRNA against OGG1 to inhibit its expression. Then, these cells were exposed to 900 MHz RF-EMFs radiation. The results of the FPG-modified alkaline comet assay showed significant exacerbation of DNA base damage at SARs of 2 W/kg and 1 W/kg. Therefore, we demonstrated a critical role of OGG1 in protecting against RF-EMFs-induced oxidative DNA base damage and RF-EMFs could even induce DNA base damage at lower SAR of 1 W/kg without OGG1. Previous similar studies have indicated that down regulating OGG1 leads to greatly exacerbated xanthine oxidase-induced mtDNA damage [67] and oxidant-induced apoptosis [67, 68]. Some studies have also indicated that substantially more severe oxidative lesions occur in OGG1-deficient mice both *in vivo* [66] and *in vitro* [32]. Up regulating or over expressing OGG1 has been shown to protect both nuclear DNA [69] and mtDNA [70, 71] from oxidative damage and to suppress cytotoxicity in response to a variety of oxidative stresses. These consistent results imply the pivotal role of OGG1 in the defense against oxidative DNA lesions. Cells isolated from OGG1-null mice are deficient in the repair of 8-oxoG [72], and cellular extracts from these mice lack the ability to excise 8-oxoG from double-stranded DNA [73], strongly suggesting that base excision by OGG1 is the major, and apparently the only, physiologically relevant mechanism for the removal of 8-oxoG from nuclear DNA in mammalian cells. As above mentioned, the damaged DNA base would be recognized by OGG1, and nicked by APE1 to form SSBs which could be detected by alkaline comet assay, however, we found that RF-EMFs exposure at 2 W/kg does not lead to DNA strand breakage in the absence of OGG1 inhibition, which may be attributed to the rapid repair of APE1 mediated single stand breaks or action of several enzymes in the induction of oxidative DNA damage[66, 74, 75]. However, in comparison to nuclear DNA, mtDNA is considerably more sensitive to oxidative damage [76, 77] and in fact, nuclear DNA is less sensitive to ROS generation [77]. Therefore, further studies are needed to identify the primary target DNA (nuclear DNA and/or mtDNA) on which OGG1 functions and the exact mechanisms by which OGG1 protects against RF-EMFs-induced DNA oxidative lesion formation.

In summary, this study demonstrated that the down-regulation of OGG1 expression apparently exacerbates RF-EMFs-induced DNA base damage in Neuro-2a cells, indicating that OGG1 plays a critical role in protecting cells against RF-EMFs exposure-induced DNA base damage. This role of OGG1 may provide insight into the molecules and substances in cells defensing against the hazardous biological effects of RF-EMFs. In addition, the data regarding oxidative DNA lesion formation suggested that OGG1 might be a potential target for preventing RF-EMFs-induced genotoxicity. This study indicates a need to develop effective tools to decrease the dose and the duration of RF-EMFs exposure in daily life and to improve the quality of the defense systems in the body against potential RF-EMFs-induced damage.

Acknowledgments

This work was supported by the National Basic Research Program of China (National 973 Program; Grant No. 2011CB503700), and the grant from the National Natural Science Foundation of China (Grant No. 31370833, 31470823).

Disclosure Statement

There are no conflicts of interest.

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