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1.7 GHz long-term evolution radiofrequency electromagnetic field with stable power monitoring and efficient thermal control has no effect on the proliferation of various human cell types

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Abstract

Long-term evolution (LTE) radiofrequency electromagnetic field (RF-EMF) is widely used in communication technologies. Thus, the influence of RF-EMF on biological systems is a major public concern and its physiological effects remain controversial. In our previous study, we showed that continuous exposure of various human cell types to 1.7 GHz LTE RF-EMF at a specific absorption rate (SAR) of 2 W/Kg for 72 h can induce cellular senescence. To understand the precise cellular effects of LTE RF-EMF, we elaborated the 1.7 GHz RF-EMF cell exposure system used in the previous study by replacing the RF signal generator and developing a software-based feedback system to improve the exposure power stability. This refinement of the 1.7 GHz LTE RF-EMF generator facilitated the automatic regulation of RF-EMF exposure, maintaining target power levels within a 3% range and a constant temperature even during the 72-h-exposure period. With the improved experimental setup, we examined the effect of continuous exposure to 1.7 GHz LTE RF-EMF at up to SAR of 8 W/Kg in human adipose tissue-derived stem cells (ASCs), Huh7, HeLa, and rat B103 cells. Surprisingly, the proliferation of all cell types, which displayed different growth rates, did not change significantly compared with that of the unexposed controls. Also, neither DNA damage nor cell cycle perturbation was observed in the 1.7 GHz LTE RF-EMF-exposed cells. However, when the thermal control system was turned off and the subsequent temperature increase induced by the RF-EMF was not controlled during continuous exposure to SAR of 8 W/Kg LTE RF-EMF, cellular proliferation increased by 35.2% at the maximum. These observations strongly suggest that the cellular effects attributed to 1.7 GHz LTE RF-EMF exposure are primarily due to the induced thermal changes rather than the RF-EMF exposure itself.

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Abbreviations: LTE, long-term evolution; RF-EMF, radiofrequency-electromagnetic field; ASC, adipose tissue-derived stem cell; SAR, specific absorption rate.

Introduction

Radiofrequency electromagnetic fields (RF-EMFs) are universally used in telecommunications and have become a daily necessity. In telecommunication technologies, long-term evolution (LTE) technology in the 1.7 to 1.8 GHz is widely used in the 4th generation mobile technologies, especially in Korea [1, 2]. As LTE technologies have enabled the convergence of wired and wireless networks, such as GSM, LAN, and Bluetooth [3–5], LTE is currently the most widely adopted telecommunication technology. Despite the extensive daily use of these technologies, the physiological effects of LTE RF-EMFs on humans are not fully understood. However, these physiological effects are a major public health concern.

The International Commission on Non-Ionizing Radiation Protection (ICNIRP) defines a specific absorption rate (SAR) of 2 W/kg for the local head/torso and 0.08 W/kg for the whole body as the safety limits for mobile device emission in the general public [6]; however, this limit remains controversial. Several studies have revealed the adverse effects of 1.8 GHz RF-EMF in human and mouse cells such as induction of DNA breakage and oxidative stress. Intermittent exposure (5 min on/10 min off) to a SAR of 2 W/Kg 1.8 GHz RF-EMF for 24 h induced DNA single- and double-strand breaks in human fibroblasts and transformed GFSH-R17 rat granulosa cells [7]. In addition, 1.8 GHz RF-EMF exposure at a SAR of 4 W/Kg for 24 h led to oxidative DNA damage in mouse spermatocyte-derived GC-2 cells [8]. Xu *et al*. revealed that oxidative stress induced by exposure to a SAR of 2 W/Kg 1.8 GHz RF-EMF might damage mitochondrial DNA, resulting in neurotoxicity [9]. However, these researchers also demonstrated that exposure to 1.8 GHz RF-EMF at a SAR of 4 W/Kg did not elicit DNA damage [10]. Li *et al*. reported that exposures to 1.8 GHz RF-EMF at a SAR of 4 W/kg for 48 h did not affect cell viability but impaired neurite outgrowth in primary mouse hippocampal neurons and Neuro2a cells [11]

Other studies have reported that RF-EMFs have no effects on mitochondrial function and did not induce apoptosis or chromosomal alterations; exposure to 1.95 GHz RF-EMF ranging from 0 to 4 W/Kg for up to 66 h did not induce apoptosis, oxidative stress, or DNA damage in human hematopoietic stem cells or the human leukemia cell line, HL-60 [12]. Intermittent exposure (5 min on/30 min off) to a SAR of 1.5 W/Kg 1.71 GHz RF-EMF did not induce any significant cellular dysfunction in mouse embryonic stem cell-derived neural progenitor cells [13]. In addition, exposure to 900 MHz at 40 V/m for 1 h had no significant effect on the viability of human epidermal keratinocytes [14]. Exposure to 27.1 MHz RF-EMF did not influence the viability of the human keratinocyte cell line, HaCaT [15]. Thus, the effect of RF-EMF on cellular physiology remains controversial, and this uncertainty is another reason for public fear.

To understand the effects of RF-EMF in model organisms, the US National Toxicology Program (NTP) and the Ramazzini Institute in Italy conducted a carcinogenicity study of base-station exposure in mice and rats for more than 2 years. According to their findings, 900 MHz RF-EMF increased the incidence of heart Schwannoma in rats [16, 17]. However, the ICNIRP highlighted substantial limitations of the statistical analyses and stated that these loopholes preclude the conclusions drawn concerning RF-EMF and its carcinogenesis [18]. A systematic analysis of 27 *in vivo* studies on the carcinogenic effect of RF-EMF exposure demonstrated low or inadequate evidence for an association between RF exposure and the onset of neoplasm in all tissues, although an increased risk of malignant tumors was observed in heart, brain, and intestine [19]. Owing to these controversies, continuous follow-up studies are required to identify the physiological changes induced by RF-EMF at both the cell and organism levels.

In our previous study, continuous exposure to a SAR of 2 W/Kg 1.7 GHz LTE RF-EMF for 72 h inhibited the proliferation of various human cells by inducing cell senescence [20].

Consistent with our result, using the same 1.7 GHz LTE RF-EMF generator employed by Choi *et al.* [20], Kim *et al.* reported that the proliferation of SH-SY5Y cells was significantly decreased upon exposure at a SAR of 4 W/kg for 4 h/day for 4 days by inducing cellular senescence [21]. With the same LTE RF-EMF generator employed by Choi *et al.* [20], we also observed that exposure to SAR of 0.4 W/Kg 1.7 GHz LTE RF-EMF for 24 h activated cell proliferation, while exposure to SAR of 4 W/Kg decreased the proliferation of human ASCs and Huh7 hepatocarcinoma cells (S1A and S1B Fig).

In this study, we elaborated the 1.7 GHz LTE RF-EMF cell exposure system previously used in Choi *et al.* [20] and Kim *et al.* [21] by stabilizing the power control and eliminating the thermal effect. Using this refined RF-EMF exposure system, we aimed to understand the precise physiological effect of 1.7 GHz LTE RF-EMF on the proliferation of various human cell types with different growth rates.

Materials and methods

Sources of cells and culture

Human adenocarcinoma HeLa cells were purchased from the American Type Culture Collection, and human hepatocellular carcinoma Huh7 cells were purchased from the Korean Cell Line Bank. Human ASCs were purchased from Thermo Fisher Scientific (Waltham, MA, USA). B103 rat neuroblastoma cells were a gift from Dr. Inhee Mook-Jung (Seoul National University College of Medicine, Seoul, Korea).

HeLa, B103, and Huh7 cells were cultured in high glucose-containing Dulbecco's modified Eagle's medium (DMEM; Gibco) supplemented with 10% fetal bovine serum (FBS; Sigma-Aldrich, St. Louis, MO, USA) and 1% penicillin-streptomycin (Gibco). ASCs were grown in DMEM/F12 (Gibco) supplemented with 10% FBS and 1% penicillin-streptomycin. All cell types were cultured at 37˚C in a humidified atmosphere containing 5% CO_2.

Cell exposure to the LTE RF-EMF radiation system

The exposure system was preheated for a minimum of 30 min before 1.7 GHz LTE RF-EMF exposure. A total of 30×10^4 ASCs and 20×10^4 Huh7, HeLa, and B103 cells were seeded and incubated in a 100-mm dish for 16 h before RF-EMF exposure. For RF-EMF exposure, as previously described [20], 100 mm culture dishes were placed 13.6 cm from the conical antenna, which was located at the center of the exposure chamber. The cells in the dishes were then exposed to the RF-EMF of a single LTE signal at SAR values ranging from 0.4 to 8 W/Kg for the described duration. During the exposure, the temperature of the exposure chamber was maintained at 37 ± 0.5˚C by circulating water within the chamber. The unexposed sham group was incubated under the same conditions without RF-EMF exposure. After RF-EMF exposure, the cells were counted using Cellometer Auto T4 (Nexcelom) and used for further assays.

Cell viability assay

Cell viability was monitored using cell counting and MTT assays. After exposure to RF-EMF for the indicated time periods, the cells were harvested and counted using a Cellometer Auto T4 (Nexcelom). For the MTT assay, Huh7, HeLa, and B103 cells were incubated in 6 mL of medium containing 0.5% 3-(4,5-dimethylthiazol-2-yl)-2,5-di-phenyltetrazolium bromide (MTT; Amresco Inc., OH, USA) at 37˚C for 90 min and ASCs for 3 h. The resulting formazan crystals were dissolved in 6 mL of dimethyl sulfoxide (DMSO), and the optical density was measured at 570 nm using an ELISA microplate reader (SpectraMax ABS, Molecular Device Co., CA, USA).

Western blot analysis

After exposure to RF-EMF, cells were harvested, and protein lysates were prepared as previously described [20]. Histones were extracted with 0.2 M HCl and neutralized with 1 M NaOH. Lysates or histones with 15–20 μg of protein were separated on 10–15% SDS polyacrylamide gels (PAGE) and electro-transferred to PVDF membranes (Merck Millipore, Billerica, MA, USA). The membranes were incubated overnight at 4˚C with primary antibodies including anti-phospho-Erk1/2, anti-Erk1/2, anti- β-actin, anti-histone3 (Cell signaling Technology, Inc.), and anti-phospho-H2AX (Millipore, Germany). After primary antibody incubation, membranes were then treated with the appropriate secondary antibodies for 1 h at room temperature. Protein bands were visualized by chemiluminescence detected with AmershamTM ImageQuant 800TM (Cytiva).

Flow cytometry

HeLa, B103, and ASC cells exposed to RF-EMF for 72 h were harvested for each assay. To examine the apoptotic cell population, cells were resuspended in 1X Annexin V binding buffer and incubated with FITC conjugated Annexin V and propidium iodide (PI; BD Pharmingen™). For cell cycle analysis, cells exposed to RF-EMF for 72 h were pulsed with 10 μM 5-bromo-2'-deoxyuridine (BrdU) for 30 min and stained with FITC BrdU Flow Kit after fixed (BD Pharmingen™). After staining, at least 10,000 cells per assay were analyzed using BD Accuri™ C6 Plus (BD Bioscience) with FlowJo V10 software (Tree Star, San Carlos, CA, USA).

Statistical analysis

All statistical analyses were performed using the Mann-Whitney U test in GraphPad Prism 9 (GraphPad Software Inc., San Diego, CA, USA). All data are presented as mean ± standard deviation (SD) of more than three independent experiments with statistical significance. $P<0.05$ (*), $P<0.01$ (**), and $P<0.001$ (***) were considered to indicate statistical significance while $P>0.05$ was considered to indicate statistical non-significance (ns).

Results

A new RF-EMF signal generator and a software-based target power feedback system increase the stability of 1.7 GHz LTE RF-EMF signal generation

The detailed information on the 1.76 GHz LTE cell exposure device was mainly described by Choi *et al.* [20]. This *in vitro* exposure device used a radial transmission line (RTL) exposure system to expose multiple dishes of cells simultaneously. The 1.76 GHz LTE signal was applied to the RTL exposure system after amplification. The maximum input power was 60 W. The input signal was fed through a conical antenna with broadband characteristics. The external dimensions of the exposure system were 843 mm x 825 mm x 315 mm. It consists of an incubator, water circulator, signal generator, power meter, power amplifier, and control computer (Fig 1A). A JS-CO2-AT-750 incubator (John Sam Corp.) was used to maintain a controlled environment for the cell culture, and a C-WBL water circulator (Chang-shin Science) was used to provide cooling and eliminate any thermal effects during exposure.

To refine the system, a new signal generator, E4438C (Keysight Technologies), was employed to generate the LTE signal. The generated LTE signal was amplified using a customized power amplifier developed to ensure a maximum output power of 60 W, and an E4418B power meter (Keysight Technologies) was employed to measure the amplifier output powers (Fig 1A). To continuously monitor the power generated by E4418B, a customized control software was developed and installed on a PC to display the power measurement results (Fig 1B). During the exposure,

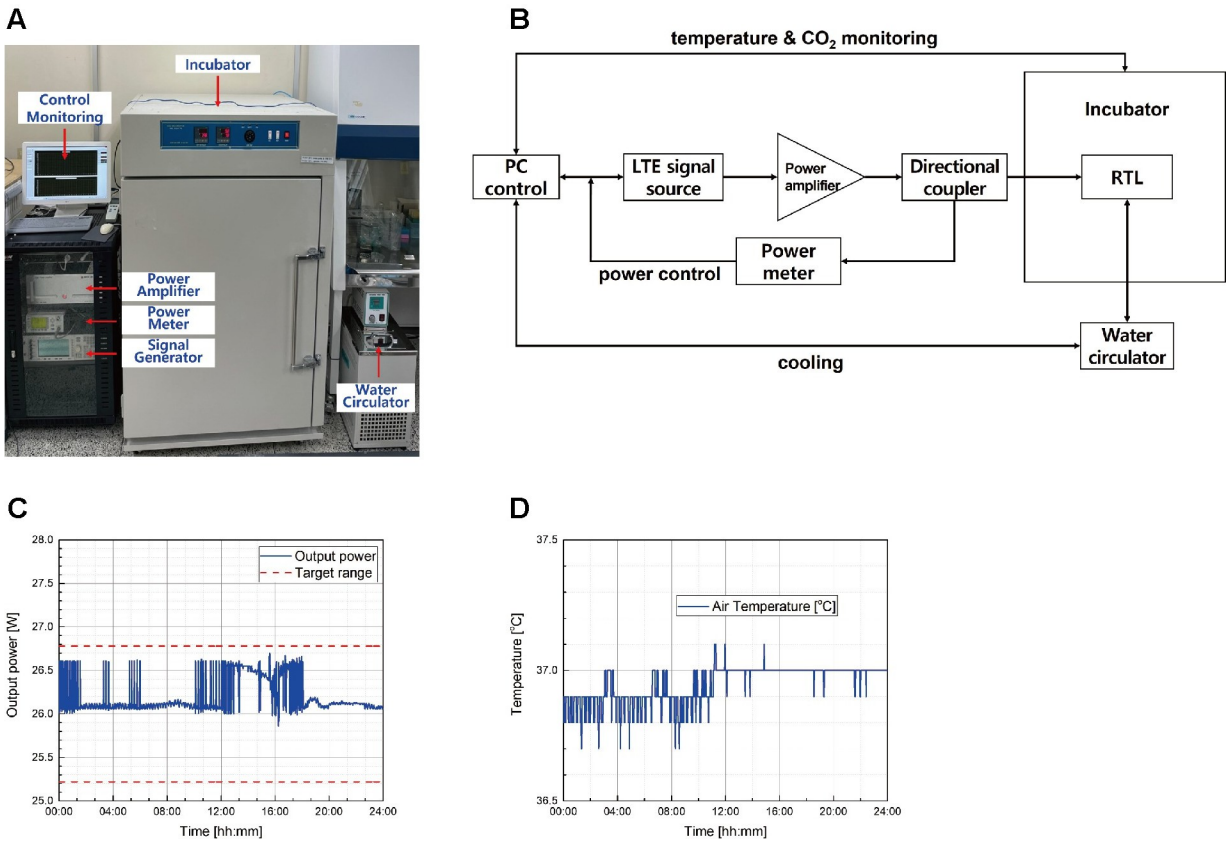

Fig 1. The elaborate 1.7 GHz LTE RF-EMF cell exposure system used in this study. (A) An image of the 1.7 GHz RF-EMF exposure device. (B) The block diagram of the 1.7 GHz LTE RF-EMF exposure system. (C) The output power was monitored for 24 h. (D) The air temperature of the incubator was monitored for 24 h.

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the PC continuously recorded all experimental data and controlled all feedback flows to regulate the desired conditions and settings in real time. All exposure conditions, such as frequency, duration, and signal level, can be defined using the main software. The LTE signal was amplified to a desired level using a power amplifier and injected into a radial transmission line. The power measured through a directional coupler and a power meter functions as a feedback loop connected to an LTE signal generator, which maintains the power within ± 3% of the target output power level. This feedback scheme was implemented to regulate the one-minute average output power, which is crucial for maintaining the required SAR values in a stable manner.

To check the stability of the output power through power control, we set the target power to 26 W and the target range to ± 3%, and then monitored the output for 24 h. An example of power monitoring is presented in Fig 1C, which shows that the output power was well controlled within the target range (Fig 1C). The mean output was 26.12 W, and the standard deviation was 0.76%. The air temperature in the incubator was also well controlled, with a mean of 36.95˚C (Fig 1D).

Exposure to the 1.7 GHz LTE RF-EMF generated with the refined system did not affect the proliferation of various mammalian cells

As described in Materials and Method, Huh7, HeLa, and B103 cells were seeded and incubated for 16 h before being exposed to RF-EMF. We previously reported that exposure to 1.7 GHz

LTE RF-EMF at a SAR of 2 W/Kg for 72 h induced senescence in ASCs and Huh7 cells [20]. In addition, the number of ASCs and Huh7 cells decreased by 7% and 20%, respectively, following exposure to the same system at a SAR of 4 W/Kg for 24 h (S1B Fig). Using the same experimental setup, when ASCs and Huh7 cells were exposed to SAR of 0.4 W/Kg for 24 h and incubated for an additional 48 h, the number of ASCs and Huh7 cells increased by 27% and 37%, respectively, compared with that of the unexposed controls (S1A Fig). However, with the new elaborate RF-EMF exposure equipment setup, under the same exposure conditions used in the previous setup (24 h of exposure followed by 48 h of incubation), neither ASCs nor Huh7 cells showed any significant change in cell number after exposure to SAR of 0.4 W/Kg and 4 W/Kg, compared to the unexposed controls (Fig 2A).

To assess the effects of exposure to the 1.7 GHz LTE RF-EMF generated using this refined system on cell proliferation, we extended the exposure time to 72 h with the exposure intensity ranging from SAR of 0.4 W/Kg to 8 W/Kg and evaluated cell viability by MTT assays. If the exposure to RF-EMF positively or negatively affects cell proliferation, the effect would be more obvious in fast-growing cells. Thus, we first examined the actual doubling time of each cell type used in this study: 18 h for rat neuroblastoma B103, 25 h for HeLa, 28 h for Huh7, and 34 h for ASC. The doubling time of each cell type showed that the rat cell grows faster than human cells and that human cancer cells grow faster than the normal stem cells as reported [22]. We used the fast-growing human cancer cells, Huh7 and HeLa, for the exposure and viability assays. We also exposed LTE RF-EMF to B103 rat neuroblastoma cells, which grow faster than human cancer cells. Only the effect of RF-EMF exposure at SAR of 8 W/Kg was monitored in ASCs because ASCs usually proliferate slower than cancer cells and exposure effects would not be obvious at low intensity. The viability of Huh7, HeLa, and B103 cells exposed to 1.7 GHz LTE RF-EMF was not significantly different from that of the unexposed controls at all tested SAR values (Fig 2B and 2C). Correspondingly, at SAR of 8 W/Kg, no significant difference was noted in the viability of exposed ASCs compared with that of the unexposed control (Fig 2D). Thus, to further verify that there is no effect from exposure to 1.7 GHz LTE RF-EMF on various human cells, we used the strongest exposure condition, a continuous 72 h exposure with 8 W/Kg.

Exposure to 1.7 GHz LTE RF-EMF generated with the refined system did not induce DNA damage, cell cycle modulation, and apoptotic cell death

To confirm the result of MTT assays that 1.7 GHz RF-EMF did not affect cellular proliferation in various cell types, we also analyzed the apoptotic cells and the distribution of cells in cell cycle stages by flow cytometry with each cell type, after cells were exposed to LTE RF-EMF at 8 SAR for 72 h. To examine the apoptotic cell population, cells were stained with FITC-conjugated Annexin V and propidium iodide (PI). No apoptotic population of cells was observed in the RF-EMF-exposed HeLa, ASC, and B103 cells as in the un-exposed negative controls, while the UV-exposed cells of each type showed apoptotic populations (Fig 3A). Distribution of cells in the cell cycle stages was also examined in the RF-EMF-exposed cells after BrdU incorporation. No significant difference in the cell cycle distribution was observed between the exposed cells and the unexposed controls, suggesting that the RF-EMF exposure did not delay or accelerate the cell cycle progression (Fig 3B). We then examined the activation of ERK signaling pathway that is usually triggered in response to various stresses. The phosphorylated ERK and the amount of ERK in the RF-EMF-exposed cells and the unexposed controls were detected. Consistent with the cell cycle analyses, we could not observe any significant difference in the expression level of phosphorylated ERK and ERK of the RF-EMF-exposed and of the unexposed control cells (Fig 3C). To support these results, we monitored DNA damage in

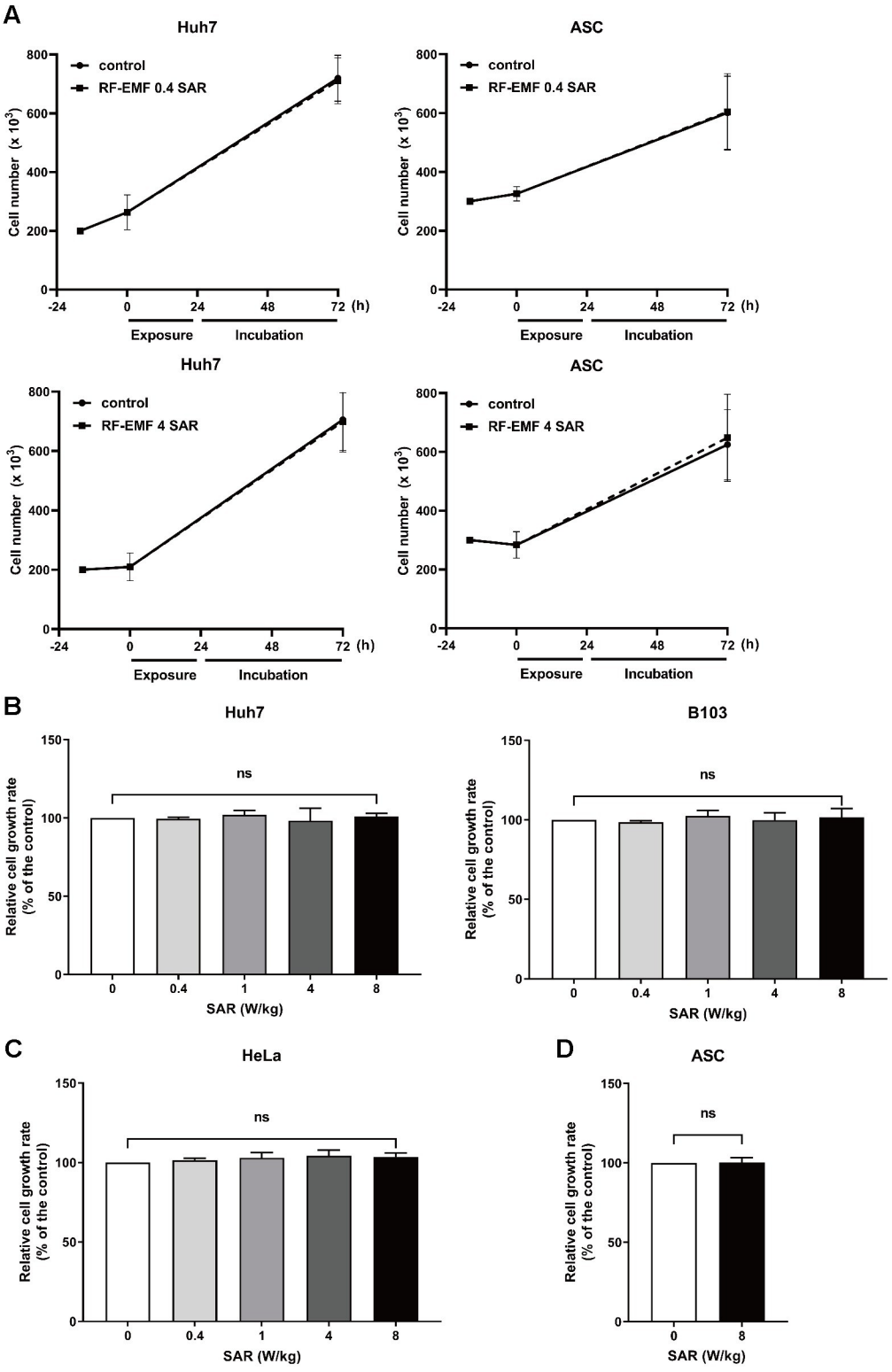

Fig 2. The exposure of various mammalian cells to 1.7 GHz LTE RF-EMF generated in this refined system did not affect cellular proliferation. The same number of cells was seeded and incubated for 16 h. (A) Huh7 cells and ASCs were exposed to the SAR of 0.4 W/Kg or 4 W/Kg RF-EMF for 24 h and further incubated for 48 h without RF-EMF exposure. After incubation, the cells were counted using a cell counter. (B) Huh7, HeLa, and (C) B103 cells were exposed to RF-EMF for 72 h at the indicated SAR values. After the exposure, cell viability was assessed using the MTT

assay. (D) ASCs were exposed to the SAR of 8 W/Kg RF-EMF for 72 h, and cell viability was assessed using the MTT assay. Zero (0) SAR: Unexposed controls. More than three independent replicates were tested in all experiments, and the graph represents the pooled data from three independent experiments. Data were analyzed with Mann-Whitney U test and presented as mean ± SD. ns represents non-significant.

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the LTE RF-EMF-exposed cells with double-strand break marker phospho-histone 2AX (γ-H2AX). Consistently, no γ-H2AX was observed between the exposed cells and the unexposed control groups, while we could detect γ -H2AX in the UV-radiated cells (Fig 3D). Taken together, these observations strongly support the notion that exposure to 1.7 GHz LTE RF-EMF does not induce any DNA damage, cell cycle modulation, and apoptotic cell death.

Exposure to 1.7 GHz LTE RF-EMF without the temperature control system activated cell proliferation

We showed in this study that no cellular effect was observed by the exposure to 1.7 GHz LTE RF-EMF generated with our new elaborate system, while pro- and anti-proliferative effects of 1.7 GHz LTE RF-EMF were detected depending on the SAR values with our previous generator setup. Thus, we speculate that the temperature elevation caused by RF-EMF generation in the previous setup might be responsible for the observed cellular effects of the RF-EMF. To confirm this speculation, we monitored the temperature of the exposure chamber as well as cell media in the chamber and evaluated the cell viability without turning-on the water circulation system. First, we turned on the 1.7 GHz LTE RF-EMF generator at the SAR of 8 W/Kg for 72 h without water circulation and recorded the temperature of the exposure chamber and the media in the chamber. The chamber temperature increased by approximately 1.7˚C with turning-off the water circulator, compared to that obtained when the circulator was turned on (Fig 4A). Additionally, the temperature of the media in the chamber heightened by approximately 1.8˚C. When B103 and HeLa cells and ASCs were exposed to 1.7 GHz LTE RF-EMF at the SAR of 8 W/Kg without maintaining the temperature by water circulation, we observed a significant acceleration in cell proliferation depending on the cell growth rate: 11% for ASCs, 24.3% for HeLa, and 35.2% for B103 (Fig 4B). Proliferation was increased more effectively in the fast-growing B103 cells and less effectively in the slow-growing ASCs.

Discussion

We showed in this study that 1.7 GHz LTE RF-EMF from a stabilized generating system with minimal thermal effect has no effect on the viability, cell cycle, stress response signaling pathway, and DNA damage of various mammalian cell types with different growth rates. However, when the temperature was not adequately controlled, cell proliferation was promoted upon exposure to RF-EMF. These observations strongly suggest that temperature is a major underlying factor for the previously reported cellular effects of 1.7 GHz LTE RF-EMF.

Temperature is a well-established factor that influences various cellular processes, including metabolic activity and enzymatic reactions. Various biological species exhibit a temperature-dependent increase in cell proliferation within specific temperature ranges [23]. For instance, HeLa cells exhibit a temperature-dependent increase in growth rate between 33 and 38˚C [24]. Conversely, temperatures outside a certain range can have adverse effects on cell growth, inducing cold- or heat-shock responses. Heat shock stress induces the unfolding of intracellular proteins, as well as cytoskeleton and cell membrane damage. Consequently, accumulation of heat stress can lead to cell cycle arrest or cell death [25]. Furthermore, heat stress-induced alterations in the mitochondrial antioxidant system can result in increased ROS generation [26].

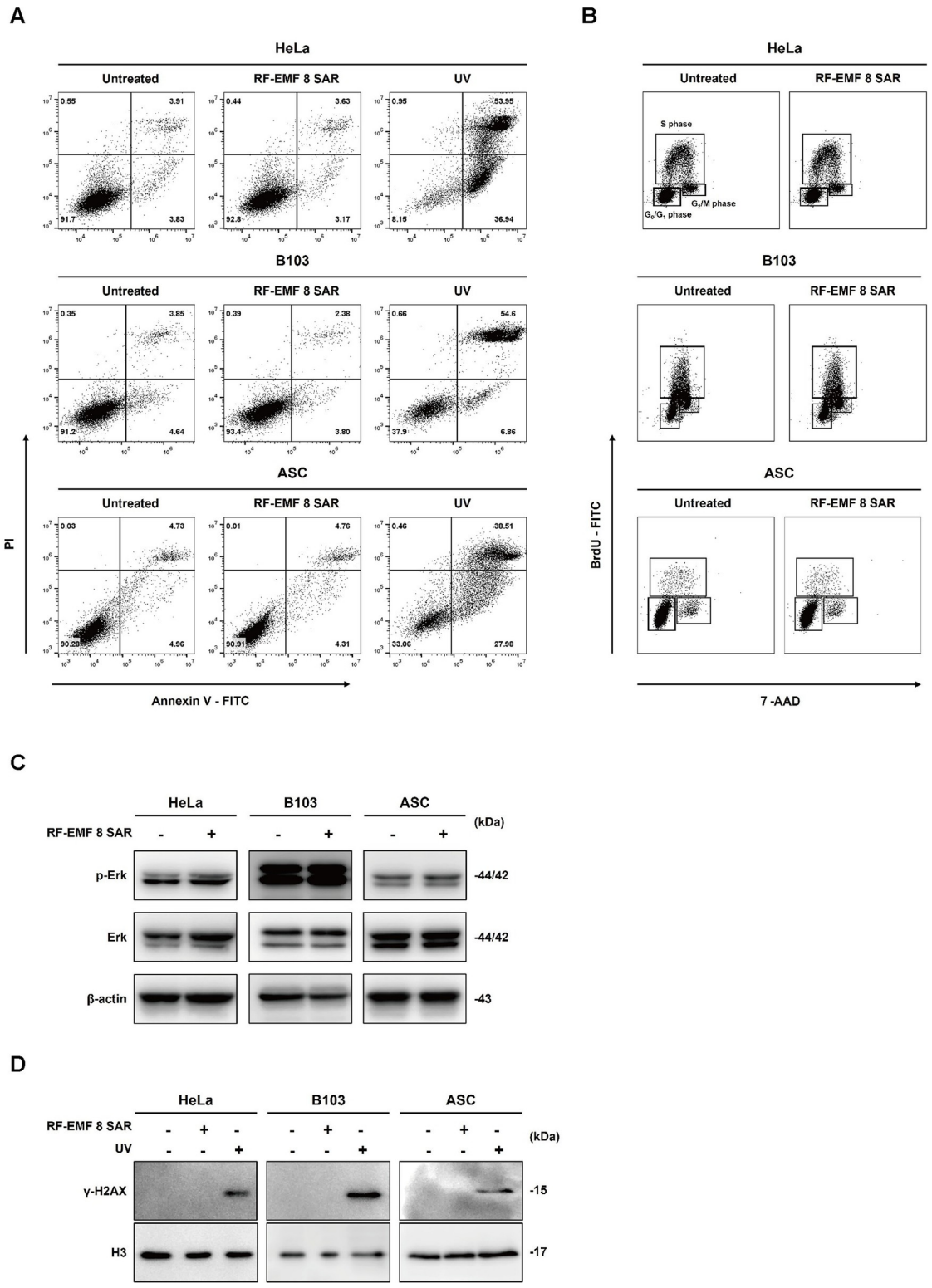

Fig 3. Neither cell cycle perturbation nor DNA damage was induced by 1.7 GHz LTE RF-EMF generated in this refined system. The same number of cells was seeded and incubated for 16 h. Then, (A) the cells of HeLa, B103, and ASCs were exposed to the SAR of 8W/Kg RF-EMF for 72 h and double stained with Annexin V-FITC and PI. The cells of each kind exposed to UV for 30 min in the PBS were used as a positive control for apoptosis. Stained cells were analyzed with flow cytometry and presented as dot plots. (B) The cells were double stained with BrdU-FITC and 7-AAD and analyzed by flow cytometry. Cell cycle stage proportion was

presented with dot plots. (C) p-ERK and ERK were detected by western blot analysis in RF-EMF exposed and unexposed cells. β-actin served as a loading control. (D) Histones were extracted from cells and γ -H2AX was detected by western blot analysis. Histone H3 served as a loading control.

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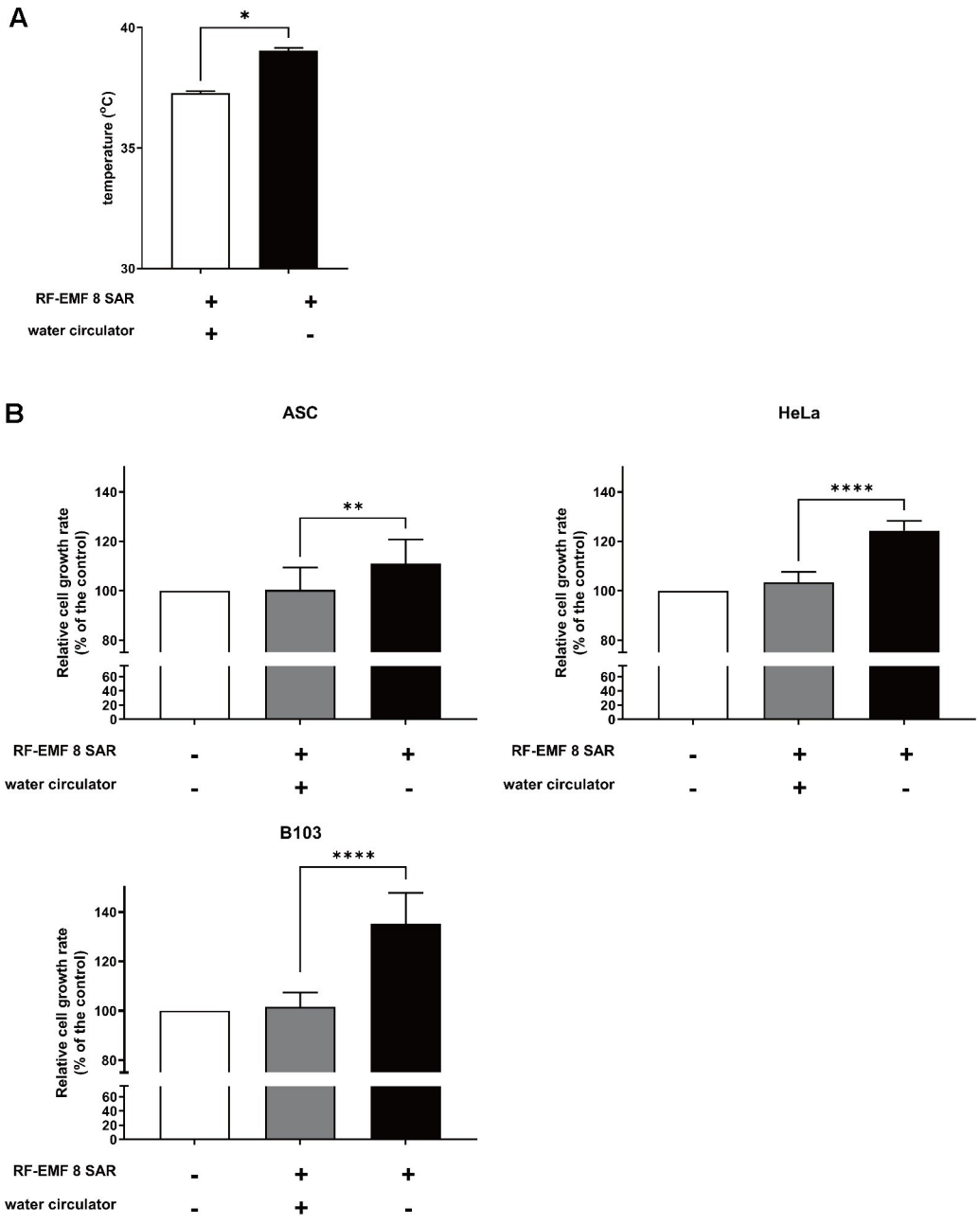

Fig 4. The exposure of various mammalian cells to 1.7 GHz LTE RF-EMF without heat control activated cell proliferation due to thermal effects. (A) During turning-on 1.7 GHz LTE RF-EMF with the SAR of 8 W/Kg, the temperature within the exposure chamber was recorded every minute for 72 h, and the average temperature was calculated. The temperature was measured when the water circulator was turned on or off. (B) The same number of HeLa, B103, and ASCs were seeded for 16 h and exposed to the SAR of 8 W/Kg 1.7 GHz RF-EMF for 72 h, while the water circulator was turned on or off. Cell proliferation was measured using the MTT assay. More than three independent replicates were tested in all experiments, and the graphs represent the pooled data from three independent experiments. Data were analyzed with Mann-Whitney U test and presented as mean ± SD. $P < 0.0001$ (****), $P < 0.01$ (**).

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As RF-EMF leads to temperature elevation, investigation of the direct effects of RF-EMF on cells and biological organisms requires precise temperature control and monitoring. In our case, switching the RF-EMF generator and implementing a feedback monitoring system enabled more consistent generation of LTE RF-EMF with stable intensity. As a result, stabilization of the intensity contributes to an increase in thermal stability. Using this improved RF-EMF exposure system, we verified that the anti- and pro-proliferative effects observed and reported in our previous studies were mainly due to thermal effects.

Our study critically presents the importance of using an adequate exposure system when investigating the biological effects of RF-EMF. One of the reasons for the different physiological outcomes of different research groups by the exposure to RF-EMF and related controversy over its effects might be due to the difference in exposure devices. All scientists in this field agree on the importance and necessity of a well-characterized RF exposure system. Also, there are recommended minimal requirements and development guidelines for exposure setups of bio-experiments addressing health risk concerns [27, 28]. Unfortunately, however, we do not have an internationally standardized exposure system yet. Thus, future research groups should assess the cellular or physiological effects of RF-EMF with a more elaborate RF-EMF exposure system that has better power and thermal controls.

We expect that the influence of 1.7 GHz LTE RF-EMF exposure, at least up to SAR of 8 W/Kg, on biological organisms would be minimal, as biological organisms usually have better thermal control systems for the maintenance of homeostasis. Nonetheless, our results should be confined to 1.7 GHz LTE RF-EMF, and RF-EMF with different frequencies might have different physiological outcomes.

Conclusion

To understand the precise cellular effect of 1.7 GHz LTE RF-EMF, we developed an RF-EMF cell exposure system with an improved RF signal generator and control software. With a refined RF-EMF exposure system, we could maintain a consistent target power during the 72-h-exposure period with minimal thermal effects. With this refined experimental setup, exposure to 1.7 GHz LTE RF-EMF at the SAR ranging from 0.4 W/Kg to 8 W/Kg neither affects the proliferation of various human cells with different growth rates nor induces DNA damage and cell cycle perturbation. Before upgrading the exposure system, we observed that the exposure of human cells to 1.7 GHz RF-EMF increased or decreased cell proliferation, depending on the SAR values. In addition, we verified that exposure to 1.7 GHz RF-EMF with this refined system affected cell proliferation when heat was not properly controlled. Altogether, these results suggest that exposure to 1.7 GHz LTE RF-EMF does not directly influence cell proliferation and that the physiological changes induced by RF-EMF might be associated with thermal effects.

Supporting information

S1 Fig. 1.7 GHz LTE RF-EMF generated by the previous system induced a positive or a negative effect on cellular growth depending on the SAR values. The equal number of ASCs and Huh7 cells was seeded for 16 h, exposed to the SAR of (A) 0.4 W/Kg or (B) 4 W/Kg 1.7 GHz LTE RF-EMF for 24 h, and further incubated for 48 h without RF-EMF exposure. After incubation, the cells were counted using a cell counter and plotted. Three independent experiments were performed, and the cell number is presented as mean ± SD. $P < 0.001$ (***), $P < 0.05$ (*).
(TIF)

S2 Fig.
(TIF)

S3 Fig.
(TIF)

S1 Data.
(XLSX)

Author Contributions

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Formal analysis: Kiwon Song.

Funding acquisition: Sangbong Jeon, Kiwon Song.

Investigation: Jaeseong Goh, Dongwha Suh.

Methodology: Sangbong Jeon, Youngseung Lee.

Project administration: Sangbong Jeon, Nam Kim.

Resources: Nam Kim.

Software: Sangbong Jeon, Youngseung Lee.

Supervision: Kiwon Song.

Writing – original draft: Jaeseong Goh, Sangbong Jeon, Kiwon Song.

Writing – review & editing: Kiwon Song.

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