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Radiofrequency Fields Preferentially Enhance *In Vitro* Cellular Radiosensitivity to Large Fractional Doses in a p53-Dependent Manner

Angela Chinhengo, Antonio Serafin, & John Akudugu*

Division of Radiobiology, Department of Medical Imaging and Clinical Oncology, Faculty of Medicine and Health Sciences, Stellenbosch University, South Africa

*Address all correspondence to: John Akudugu, Division of Radiobiology, Department of Medical Imaging and Clinical Oncology, Faculty of Medicine and Health Sciences, Stellenbosch University, Tygerberg 7505, South Africa; Tel.: +27-21-938-9942, E-mail: jakudugu@sun.ac.za

ABSTRACT: Hypofractionated radiotherapy, which employs large fractions of ionizing radiation, is an effective treatment modality for most superficial cancers, but may result in severe side effects from normal tissue toxicity. It is, therefore, desirable to identify radiation modifying agents that potentiate the tumor inactivating effects of ionizing radiation and thereby lead to a reduction in radiation dose and prevent normal tissue toxicity. This study assessed the effect of radiofrequency fields (RFF), modulated at 100 and 1000 Hz, on the radiosensitivity of four human cell lines: MeWo (melanoma; p53 mutant), Be11 (melanoma; p53 wild-type), DU145 (prostate cancer; p53 mutant), and L132 (normal lung fibroblasts; p53 wild-type), using the colony assay. The magnetic flux densities that were induced in cell cultures ranged from 6.74 to 22.43 μT. The data demonstrate that RFF are more efficient in modulating large fractional doses of X-rays in a frequency- and cell-type-dependent manner. Their effects on radiosensitivity also appear to be linked to p53 status, with cells with mutant p53 being less sensitized than those that are p53 wild-type. These findings suggest that RFF could find application in hypofractionated radiotherapy as adjuvants, and can have a positive impact on the treatment of superficial tumors, and specifically tumors that are p53 wild-type.

KEY WORDS: radiofrequency fields, hypofractionation, p53 status, superficial cancers

I. INTRODUCTION

Radiation therapy is a widely used cancer treatment option in the world;[1–4] however, different tumors respond differently to different radiation doses. Melanoma, sarcoma, and prostate cancer are examples of cancers that have a lower α/β ratio, making them more resistant to lower radiation doses, but sensitive to higher doses of radiation.[5] The use of hypofractionated treatment, which employs large fractional radiation doses, may be beneficial for these tumors but can pose a greater risk of normal tissue toxicity and a risk of developing severe side effects.

Considering the abovementioned challenges in radiotherapy, there is a need to find regimens that eliminate cancers with minimal invasion and reduced normal tissue toxicity or side effects. Radiofrequency fields (RFF) are a possible candidate, in combination with radiation, to sensitize tumor cells to therapeutic doses of radiation. For decades,

electromagnetic fields (EMF) have been shown to exhibit diverse biological effects and therapeutic potential on their own or in combination with other treatment modalities.[6–14] The importance of radiation modifiers in radiotherapy, radiation protection, and biological dosimetry cannot be overemphasized. Therapeutic benefit has been demonstrated for the use of electromagnetic fields in cancer patients, where EMF treatment resulted in reduced disease progression, prolonged patient survival, and no significant side effects.[15–17] In fact, significant evidence exists suggesting that electromagnetic fields could potentially be the future of noninvasive and nontoxic therapy.[18]

Although it was suggested two decades ago that EMF can enhance the effects of ionizing radiation,[19–21] there is still a paucity of studies on the combined biological effects of EMF and ionizing radiation. In a recent study by our group, no cytotoxic effects were observed in fibroblasts and melanoma cells, when cells were exposed to 27.125-MHz fields that were amplitude modulated at 100 or 1000 Hz alone.[22] However, it was demonstrated that electromagnetic fields have the desirable radiosensitizing and radioprotective effects on tumor (melanoma) and normal (fibroblasts) cells, respectively.[22] It was further shown that EMF may significantly reduce the total radiation dose during radiotherapy and minimize normal tissue toxicity without compromising on tumor control.[23] The diversity of effects, or lack thereof, is likely due to the wide range of frequencies, types of electromagnetic fields (electric, magnetic, or radio), and cellular systems used. In light of reported findings that exposure of cells to low ionizing radiation doses, similar to those used in hyperfractionated radiotherapy, may blunt the p53 response and lead to a radioadaptive response,[24] it may be prudent to use hypofractionated radiation therapy to effectively kill tumor cells without inducing a radioresistant response.[25]

To evaluate whether the radiomodulatory effects of radiofrequency fields are dependent on the fraction size of ionizing radiation and cellular p53 status, radiomodulatory effects of two RFF were assessed in human prostate cancer and melanoma cells, as well as in normal fibroblasts.

II. MATERIALS AND METHODS

A. Cell Lines and Culture Maintenance

The MeWo cell line (ATCC® Number: HTB-65™; p53 mutant) and Be11 cell line (originally isolated at the Institut Gustave Roussy, Villejuif, France[26]; p53 wild-type) are adherent, human malignant melanoma cells, and were kindly provided by Professors F. Zölzer and C. Streffer (University of Essen, Germany). The human prostate cancer cell line, DU145 (ATCC® Number: HTB-81D™; p53 mutant) is an adherent cell line derived from a metastatic lesion of the central nervous system,[27] and was a gift from Professor P. Bouic (Synexa Life Sciences, Montague Gardens, South Africa). The human normal lung epithelial cell line, L132 (ATCC® Number: CCL-5™; p53 wild-type) was a gift from Dr. T. Robson (University of Ulster, UK). L132 was used to represent normal tissue.

The cancer cells (MeWo, Be11, and DU145) were routinely cultivated in minimum essential medium (MEM), while the L132 cells were grown in Roswell Park Memorial

Institute medium (RPMI). For this, the cells were grown as monolayers in 75-cm^2 flasks, supplemented with 10% fetal bovine serum (20% for the MeWo cells) and 1% penicillin-streptomycin, and incubated at 37°C in a humidified atmosphere (95% air, 5% CO_2). Cells were used for experiments upon reaching 80–90% confluence.

For experiments, cell cultures were trypsinized and 200–15,000 cells (adjusted for radiation dose) were seeded per 25-cm^2 tissue culture flask, and left to settle for 3 h. The final volume of growth medium in each flask was 10 mL. Upon attachment, the cells were then irradiated with X-rays alone, or exposed to radiofrequency fields for 30 min prior to or following an X-ray irradiation.

B. Radiofrequency Field Generation and Exposure

For cell culture exposure to radiofrequency fields, a PERL M^+ oscillator amplifier (Resonant Light Technology Inc., Courtenay, Canada; Serial # PM 171116) was used to produce 27.12- MHz fields, square-wave amplitude modulated at 100 or 1000 Hz, with a peak-to-peak amplitude of 5 V. The modulating frequencies were generated using a ProGen II frequency generator with an output impedance of 50 Ω and a duty cycle of 50% (Resonant Light Technology, Courtenay, Canada; Serial # PG 171211), which has square and sinusoidal wave options. The resulting radiofrequencies were then broadcast via an argon-filled plasma ray tube with leaded glass electrodes [length: 29 cm; diameter: 2.55 cm; pressure: 20 Torr (100% argon)], acting as an antenna, onto the cells. The plasma ray tube operates under the principle of the Rife frequency generators,[28] which can produce tumor-specific frequencies and have gained a significant level of application in holistic medicine, over the past decades, for the treatment of many ailments, including cancer,[29,30] although their application was strongly condemned by the American Cancer Society due to scarcity of experimental evidence.[31] For sham-RFF exposure (no radiofrequency field), unirradiated (0 Gy) cell cultures were placed under the plasma ray tube when turned off. The setup is shown in Fig. 1A. A maximum of 16 cell culture flasks, stacked in groups of four, could be exposed at a given time. As illustrated in Fig. 1B, the volume occupied by the cell culture layers had outside dimensions of 11 cm (width: two flasks breadthwise) × 18 cm (length: two flasks lengthwise) × 10 cm (height: four flasks by height). The perpendicular distances from the axis of the plasma tube to the four cell culture planes were 19.0, 21.4, 23.8, and 27.0 cm. Each cell layer was covered with 10 mL of culture medium (medium depth: 3.5 mm).

The induced electric fields in the cell cultures were estimated using a large-loop H-field probe (Beehive Electronics, California, USA; cat # 100C) coupled to a digital storage oscilloscope (Hantek Electronic Co. Ltd., Qingdao, China; Serial # DSO5062BM). For this, the loop was positioned perpendicularly (in air) to the RF wave (Fig. 1B), at the respective cell culture planes. During sham exposure (background with the plasma tube turned off), the mean electric field was found to be 3.7 V/m, and was subtracted from subsequently measured peak fields when the tube was turned on. The induced peak electric fields (E_{peak}) were measured in triplicate for each modulated frequency and perpendicular distance from the plasma ray tube. No significant frequency-dependent

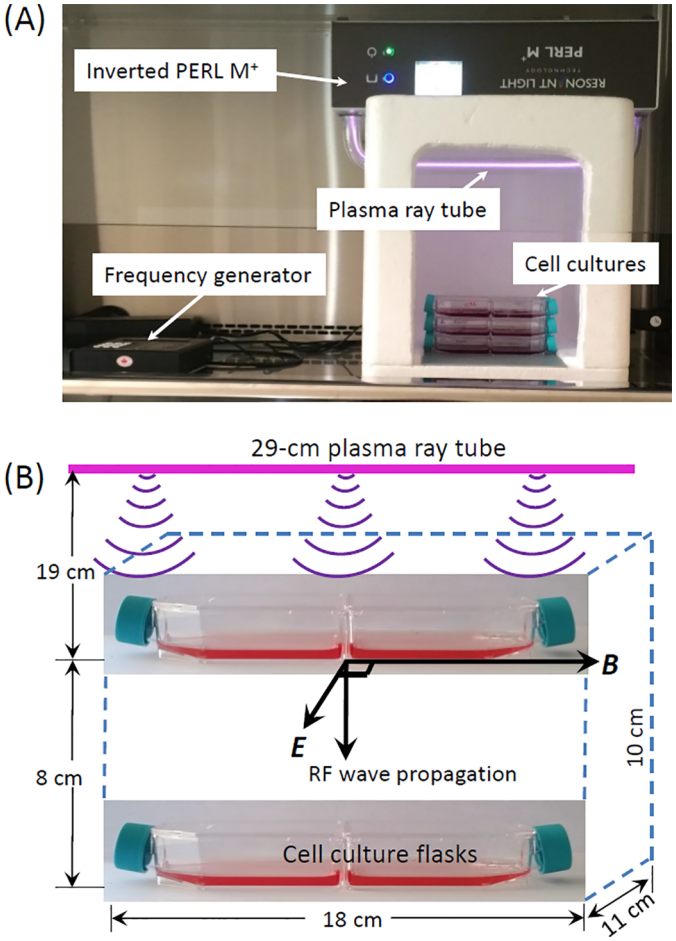

FIG. 1: (A) Photograph of the electromagnetic field (EMF) exposure system, with the PERL M^+ inverted on an appropriately cut Styrofoam box. (B) A two-dimensional schematic diagram showing the top and bottom cell culture planes of the 2 × 2 × 4 flask matrix. In the setup, the plasma ray tube is centered horizontally above the cell culture flasks, such that the induced magnetic field (B) is parallel to the base of a flask and the induced electric field (E) in the culture medium is parallel to the width of the flask.

variation in the induced electric fields was observed, and the mean of all measurements at each plane was taken as the peak electric field at that location.

The mean induced peak electric fields along the bottom of the partially filled rectangular tissue culture flasks (background subtracted) were then used to estimate the peak magnetic flux density (B, in T) from $E_{peak} = 2h\pi fB$, derived using Faraday's Law,[32] where f is the transmitted frequency (27.125×10^6 Hz), and $2h$ is the depth of the cell culture medium (0.0035 m). The uniformity of the electric field is dependent on the ratio of the depth to the width (0.05 m) of the culture medium, and diminishes as the volume (and

TABLE 1: Estimated peak electric field (*E*), magnetic flux density (*B*), and current density (*J*) induced at a distance (*d*) from the axis of a 29-cm plasma ray tube

d **(cm)**	***E*** **(V/m)**	***B*** **(μT)**	***J*** **(A/m²)**
19.0	6.69 ± 1.15	22.43	10.04
21.4	5.25 ± 0.69	17.60	7.88
23.8	3.24 ± 1.11	10.86	4.86
27.0	2.01 ± 0.51	6.74	3.02

depth) of the medium increases. Here, the ratio is less than 0.3 and the peak electric fields can be estimated to within an uncertainty of ≤ 1%.[32] The induced current densities (*J*) were also estimated from the peak electric fields, according to the relation $J = \sigma E$, assuming a conductivity (σ) of 1.5 S/m for the cell culture medium.[32] The estimated induced peak electric fields, magnetic flux density, and current density are presented in Table 1.

C. Effect of Radiofrequency Fields on Plating Efficiency

To assess if the various radiofrequencies have any cytotoxic effect on the cells, plating efficiencies determined from unirradiated cultures with no exposure to RFF were compared with those from unirradiated cultures exposed to RFF, to obtain a plating efficiency modifying factor (MF_{PE}):

$$MF_{PE} = \frac{n_{col}(0\ Gy) / n_{cell}(0\ Gy)}{n_{col}(0\ Gy + RFF) / n_{cell}(0\ Gy + RFF)}\ , \tag{1}$$

where n_{col} and n_{cell} are the number of colonies counted and cells seeded, respectively, and the ratio n_{col}/n_{cell} denotes the plating efficiency (*PE*).

D. Irradiation of Cells, Radiosensitivity, and Radiomodulatory Effects of Radiofrequency Fields

Appropriately prepared cell cultures were irradiated at room temperature (20°C) to doses up to 10 Gy, at a dose rate of 1.0 Gy/min, using a Precision MultiRad 160 X-irradiator (Precision X-Ray Inc., Branford, CT, USA). The X-ray energy and tube current were 130 kV and 23 mA, respectively. Sham-irradiated cultures (0 Gy) were left on the turntable of the running Precision X-ray irradiator for 2 min with the X-ray source turned off and were used as controls. The cell cultures were then incubated at 37°C in a humidified atmosphere (95% air, 5% CO_2) for 10 days (for DU145, Be11, and L132) and 15 days (for MeWo) for colony formation.

To terminate cultures, the growth media were decanted and colonies were washed with phosphate buffered saline, fixed in glacial acetic acid:methanol:water (1:1:8, v/v/v), stained in 0.01% amido black in fixative, washed in tap water, air-dried, and counted

using a stereoscopic microscope (Nikon, Japan; Model # SMZ-1B). Colonies containing at least 50 cells were deemed to have originated from single surviving cells and were scored. Cytotoxicity was assessed on the basis of a surviving fraction, as previously described.[22] Three independent experiments were performed for each cell line. To generate clonogenic cell survival curves, the determined mean surviving fractions (*SF*) were fitted to the linear-quadratic (LQ) model of the form

$$SF = \exp[-\alpha D - \beta D^2], \tag{2}$$

where α and β are the linear and quadratic coefficients, respectively, and *D* is the dose in Gy. Cellular radiosensitivity was expressed in terms of several indicators, namely, the surviving fraction at 2 Gy (SF_2), the surviving fraction at 6 Gy (SF_6), the mean inactivation dose ($\bar{D}$), and the α/β ratio. SF_2 and SF_6 represent low and high dose sensitivity, respectively. The mean inactivation dose, which is the area under the survival-dose response curve plotted on a linear-linear scale, represents the sensitivity over low-high doses. The α/β ratio depicts both the steepness and curvature of a survival curve, and is the dose at which the linear and quadratic components of cell killing are equal.

To investigate the influence of RFF exposure on radiosensitivity, plated cells were exposed to radiofrequency fields modulated at 100 and 1000 Hz, as described in Section B, 2 h prior to or following 1–10 Gy of X-ray irradiation. Unirradiated cultures with and without RF field exposure were used as controls for RFF and X-ray treatment, respectively. The cell cultures were then processed for colonies to form. Surviving fractions were determined for three independent experiments for each radiation dose point and frequency, and corresponding survival curves were generated. The modulatory effect of radiofrequency fields on radiosensitivity was expressed as a survival modifying factor ($MF_{survival}$), given as the ratio of surviving fractions at a dose of X-rays (or the mean inactivation dose, $\bar{D}$) in the absence and presence of RFF:

$$MF_{survival} = \frac{SF\left(X-rays\right)}{SF\left(RFF+X-rays\right)} \; or \; \frac{\bar{D}\left(X-rays\right)}{\bar{D}\left(RFF+X-rays\right)}. \tag{3}$$

The criteria for inhibition, no effect, and enhancement of radiosensitivity by RFF are $MF_{survival} < 1.0$, $MF_{survival} = 1.0$, and $MF_{survival} > 1.0$, respectively.

E. Statistical Analysis

Data analyses were performed with GraphPad Prism software (San Diego, CA, USA). All data were presented as the mean (± SEM) from three independent experiments. Where applicable, errors were determined using appropriate error propagation formulae. The unpaired two-sided *t*-test was used to compare two data sets. A $P < 0.05$ indicates a statistically significant difference between the data sets.

III. RESULTS

A. Effect of Radiofrequency Field Exposure on Plating Efficiency

To determine if radiofrequency field exposure alone affects colony forming capacity, the plating efficiencies (PE) of the cell lines in negative (medium only, PE_{medium}) and positive (RFF exposed, PE_{RFF}) controls were compared to obtain a modifying factor (MF_{PE}) as follows: $MF_{PE} = PE_{medium}/PE_{RFF}$. There was no apparent effect on the plating efficiency of all cell lines for all frequencies, with modifying factors very close to 1.0, indicating that RFF treatment alone at these frequencies is not cytotoxic. The MF_{PE} values for the 100- and 1000-Hz modulated radiofrequency fields were found to be 1.00 ± 0.03 and 0.99 ± 0.02 (for DU145), 1.08 ± 0.15 and 0.94 ± 0.03 (for MeWo), 1.03 ± 0.05 and 1.12 ± 0.06 (for Be11), and 0.94 ± 0.03 and 0.98 ± 0.04 (for L132), respectively.

B. Radiosensitivity and Radiomodulatory Effect of Radiofrequency Fields

The dose response curves for the prostate cancer cell line, DU145, are presented in Fig. 2. The radiobiological parameters are summarized in Table 2. The data show that the 100-Hz modulated field either had no effect or slightly enhanced radiosensitivity at doses between 2 and 6 Gy (Fig. 2A, Table 2).

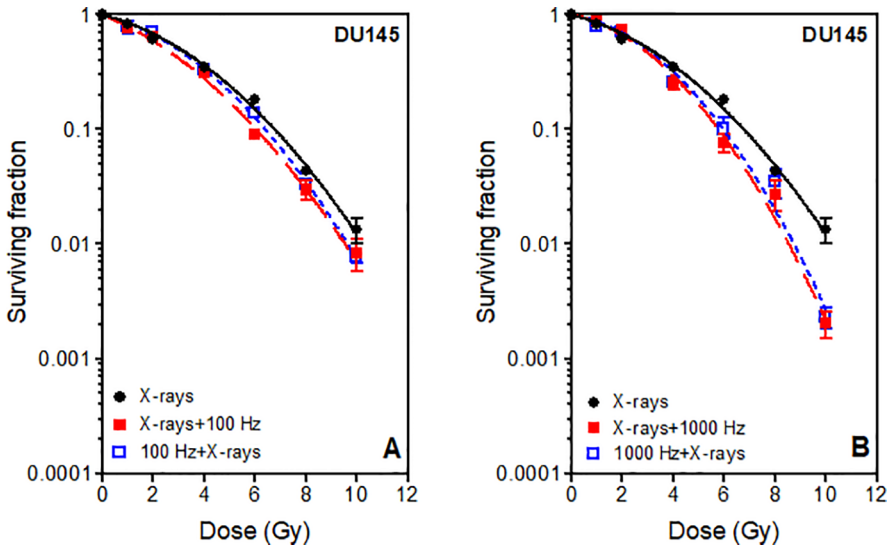

FIG. 2: Clonogenic survival curves for the DU145 cell line after X-ray irradiation alone (solid circle, solid curve) and in combination with 100-Hz (A) and 1000-Hz (B) modulated radiofrequency fields (RFF). RFF exposure was performed 2 h prior to (open square, short dashed curve) or after (solid square, long dashed curve) X-ray treatment. The survival curves were obtained by fitting data from three independent experiments to the linear-quadratic model [Eq. (2)].

TABLE 2: Summary of radiobiological parameters for the DU145 cell line. SF_2 and SF_6 denote the surviving fraction at 2 and 6 Gy, respectively. α and β are the linear and quadratic coefficients of cell inactivation, respectively. $\bar{D}$ denotes the mean inactivation dose (area under the cell survival curve). Data are presented as the mean ± SEM from three independent experiments. Modifying factors (*MF*), relative to X-ray treatment alone, derived from the SF_2, SF_6, and $\bar{D}$ values according to Eq. (3)

Treatment	SF_2	SF_6	$\bar{D}$ (Gy)	α (Gy^{-1})	β (Gy^{-2})	MF_{SF2}	MF_{SF6}	$MF_{\bar{D}}$
X-rays	0.68 ± 0.03	0.15 ± 0.01	3.44 ± 0.07	0.13 ± 0.03	0.03 ± 0.00*	—	—	—
100 Hz + X-rays	0.67 ± 0.03	0.13 ± 0.01	3.36 ± 0.11	0.13 ± 0.02	0.04 ± 0.00*	1.01 ± 0.06	1.15 ± 0.12	1.02 ± 0.04
X-rays + 100 Hz	0.59 ± 0.01	0.11 ± 0.01	3.13 ± 0.02	0.20 ± 0.03	0.03 ± 0.01	1.15 ± 0.05	1.36 ± 0.15	1.10 ± 0.02
1000 Hz + X-rays	0.69 ± 0.10	0.10 ± 0.02	3.16 ± 0.14	0.08 ± 0.06	0.06 ± 0.01	0.99 ± 0.15	1.50 ± 0.32	1.09 ± 0.05
X-rays + 1000 Hz	0.67 ± 0.09	0.09 ± 0.02	3.23 ± 0.12	0.10 ± 0.06	0.05 ± 0.01	1.02 ± 0.14	1.67 ± 0.39	1.07 ± 0.05

*Errors less than 0.01; actual values were used for propagation of errors in modifying factors.

Treatment of cells with this radiofrequency field prior to and after 2 Gy of X-rays resulted in 1% ($P = 0.8307$) and 9% ($P = 0.0621$) more cell killing, respectively. Similarly, the corresponding reductions in cell survival at 6 Gy were 2% ($P = 0.0579$) and 4% ($P = 0.0099$). The mean inactivation doses following RFF exposure before and after X-ray treatment also did not differ markedly from that for X-ray exposure alone. These data suggest that exposure of the DU145 cells to the 100-Hz modulated RFF after X-ray irradiation was more radiosensitizing than when cells were pre-exposed to this radiofrequency field, but the differences were not statistically significant ($0.0507 \leq P \leq 0.1050$). The slight sensitization, or lack thereof, is also reflected by the modifying factor not differing significantly from 1.0, as presented in Table 2.

When cells were treated with the 1000-Hz modulated field in combination with X-rays, radiosensitization was more pronounced at doses higher than 2 Gy, as shown by the much more prominent "bendiness" of combination curves compared to the X-ray only curve (Fig. 2B). At 2 Gy both pre- and post-exposure to this field did not influence the radiosensitivity, yielding modifying factors of ~ 1.0 (Table 2). On the other hand, treatment of cells with RFF prior to and after 6 Gy of X-rays resulted in 5% ($P = 0.0684$) and 6% ($P = 0.0212$) more cell killing, respectively, with corresponding modifying factors of greater than 1.0 (Table 2). Regardless of the parameter considered, there was no significant difference in radiosensitization between pre- and post-exposure to this RFF ($0.6221 \leq P \leq 0.8543$).

The data in Table 2 show that the X-rays + 100 Hz treatment yield a ~ 1.5-fold larger linear component of cell killing ($\alpha = 0.20\ Gy^{-1}$) than those for the 100 Hz + X-rays and X-ray treatments ($\alpha = 0.13\ Gy^{-1}$). The β components of cell killing for these treatments are similar. The α/β ratios for the 100 Hz + X-rays and X-rays + 100 Hz were found to be 3.25 ± 0.54 and 6.67 ± 2.44, respectively, and did not differ from that for the X-ray treatment alone (4.33 ± 1.11 Gy). However, the 1000 Hz + X-rays and X-rays + 1000 Hz treatments resulted in decreased linear and increased quadratic components of cell killing. The resulting α/β ratios were 1.33 ± 1.02 and 2.00 ± 1.26 Gy, respectively, indicating an approximately twofold reduction in ratio in the 1000-Hz modulated RFF treatment.

The cell survival curves in Fig. 3 show that pre- and post-exposure to the 100-Hz modulated radiofrequency wave had no effect on radiosensitivity in the MeWo cell line, regardless of radiation dose (Fig. 3A). Most all of the derived parameters, namely, SF_2, SF_6, $\bar{D}$, α, and β were found to be similar for both treatment sequences, giving modifying factors of approximately 1.0 (Table 3).

Pre- and post-exposure to the 1000-Hz modulated RFF led to lower cell survival at low and high doses (Fig. 3B). Treatment of the MeWo cells with this RFF prior to and after 2 Gy of X-rays led to 13% ($P = 0.0657$) and 15% ($P = 0.0277$) more cell killing, respectively (Table 3). This translates to survival modifying factors of greater than 1.0. Correspondingly, either treatment combination at 6 Gy resulted in 2% ($0.0040 \leq P \leq 0.0078$) more cell killing, yielding survival modifying factors of greater than 2.0. The mean inactivation doses for RFF exposure before and after X-ray treatment were found to be significantly lower than that for X-ray treatment alone ($0.0025 \leq P \leq 0.0191$).

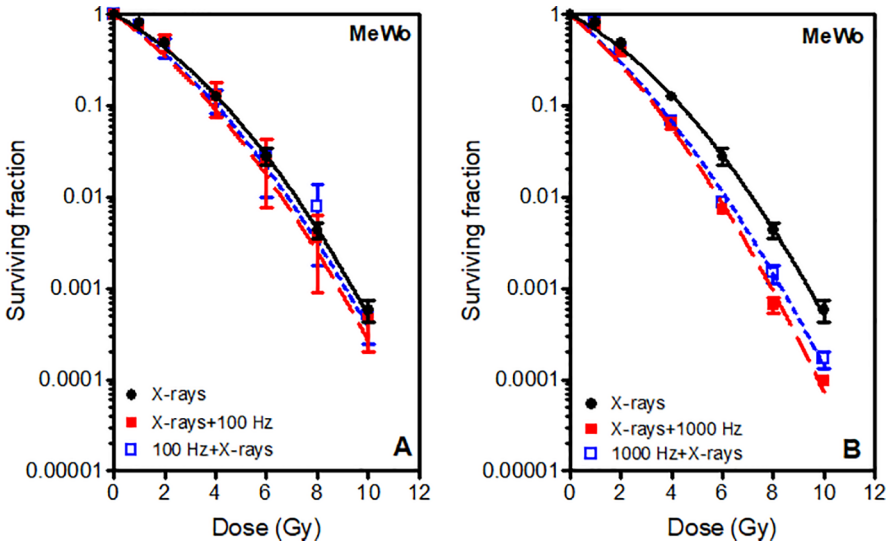

FIG. 3: Clonogenic survival curves for the MeWo cell line after X-ray irradiation alone (solid circle, solid curve) and in combination with 100-Hz (A) and 1000-Hz (B) modulated radiofrequency fields (RFF). RFF exposure was performed 2 h prior to (open square, short dashed curve) or after (solid square, long dashed curve) X-ray treatment. The survival curves were obtained by fitting data from three independent experiments to the linear-quadratic model [Eq. (2)].

However, based on all parameters, there was no statistically significant difference between RFF exposure before and after X-ray treatment ($0.1269 \leq P \leq 0.5750$).

The absence of a modulatory effect in the 100 Hz + X-rays and X-rays + 100 Hz treatment can also be due to their respective α/β ratios (10.50 ± 3.99 and 11.50 ± 6.48 Gy) not differing markedly from that of the X-ray treatment ($\alpha/\beta = 8.50 \pm 2.35$ Gy). The α coefficients for the pre-exposure and post-exposure of MeWo cells to the 1000-Hz modulated RFF were about 1.5-fold larger than that of the X-ray only treatment (Table 3). The corresponding α/β ratios were 13.25 ± 3.54 and 14.00 ± 3.72 Gy.

The survival curves in Fig. 4 show that pre- and post-exposure of Be11 cells to RFF modulated by 100 and 1000 Hz are rendered more radiosensitive compared to when the cells were exposed to X-rays only. This is evident by the much steeper (~ 4.4- to 6.9-fold larger α component of cell killing) survival curves in combination treatments compared to the survival curves from X-ray only (Table 4). The β coefficients in all cases varied over a narrow range (0.02–0.06 Gy^{-2}). The resulting α/β ratios for the 100 Hz + X-rays, X-rays + 100 Hz, 1000 Hz + X-rays, and X-rays + 1000 Hz treatments were 11.00 ± 3.55, 10.00 ± 3.91, 31.00 ± 16.01, and 30.00 ± 15.65 Gy, respectively, while that for the X-ray only treatment emerged as 1.50 ± 1.19.

The survival modifying efficacy of the 100- and 1000-Hz modulated radiofrequency fields was not affected by sequences of treatment (i.e., whether RFF exposure occurred before or after X-ray treatment), as the respective cell survival curves were congruent (Fig. 4).

TABLE 3: Summary of radiobiological parameters for the MeWo cell line. SF_2 and SF_6 denote the surviving fraction at 2 and 6 Gy, respectively. α and β are the linear and quadratic coefficients of cell inactivation, respectively. $\bar{D}$ denotes the mean inactivation dose (area under the cell survival curve). Data are presented as the mean ± SEM from three independent experiments. Modifying factors (*MF*), relative to X-ray treatment alone, derived from the SF_2, SF_6, and $\bar{D}$ values according to Eq. (3)

Treatment	SF_2	SF_6	$\bar{D}$ (Gy)	α (Gy^{-1})	β (Gy^{-2})	MF_{SF2}	MF_{SF6}	$MF_{\bar{D}}$
X-rays	0.43 ± 0.04	0.03 ± 0.00*	3.37 ± 0.03	0.34 ± 0.04	0.04 ± 0.01	—	—	—
100 Hz + X-rays	0.39 ± 0.10	0.03 ± 0.02	2.20 ± 0.31	0.42 ± 0.12	0.04 ± 0.01	1.10 ± 0.30	1.00 ± 0.56	1.53 ± 0.22
X-rays + 100 Hz	0.37 ± 0.10	0.03 ± 0.02	2.28 ± 0.32	0.46 ± 0.12	0.04 ± 0.02	1.16 ± 0.33	1.16 ± 0.71	1.48 ± 0.21
1000 Hz + X-rays	0.30 ± 0.03	0.01 ± 0.00*	2.10 ± 0.07	0.53 ± 0.05	0.04 ± 0.01	1.43 ± 0.20	2.42 ± 0.32	1.61 ± 0.06
X-rays + 1000 Hz	0.28 ± 0.02	0.01 ± 0.00*	2.01 ± 0.04	0.56 ± 0.05	0.04 ± 0.01	1.54 ± 0.18	3.22 ± 0.49	1.68 ± 0.04

*Errors less than 0.01; actual values were used for propagation of errors in modifying factors.

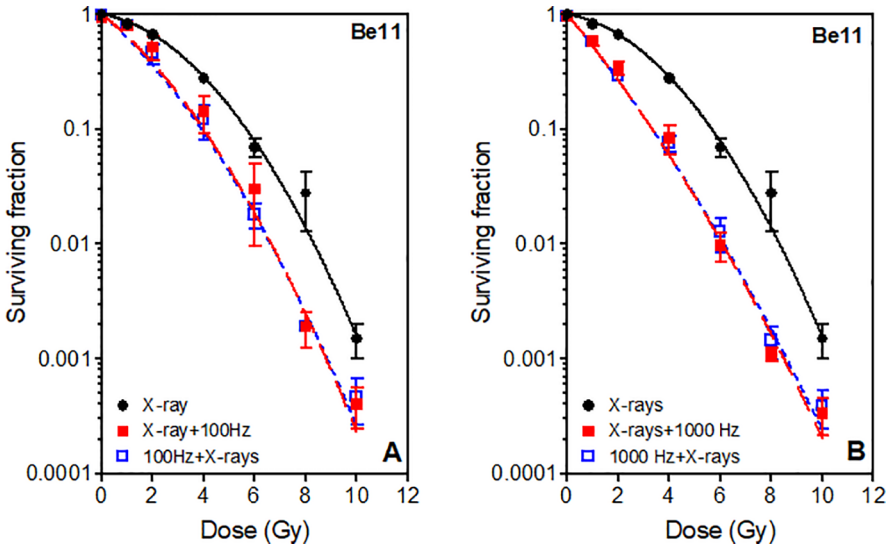

FIG. 4: Clonogenic survival curves for the Be11 cell line after X-ray irradiation alone (solid circle, solid curve) and in combination with 100-Hz (A) and 1000-Hz (B) modulated radiofrequency fields (RFF). RFF exposure was performed 2 h prior to (open square, short dashed curve) or after (solid square, long dashed curve) X-ray treatment. The survival curves were obtained by fitting data from three independent experiments to the linear-quadratic model [Eq. (2)].

Based on SF_2, SF_6, and $\bar{D}$, the 1000-Hz modulated field was ~ 1.45-, ~ 1.76-, and ~ 1.33-fold more potent in radiosensitizing the Be11 cells than the 100-Hz modulated field (Table 4).

From Fig. 5, it is apparent that both 100-Hz and 1000-Hz modulated fields have radiomodulatory effects on L132 cells, as evidenced by the steeper cell survival curves from the combination treatments when compared to that of the X-ray only treatment. Table 5 summarizes the radiobiological parameters for these treatments. Exposure to the 100-Hz modulated field prior to or after X-ray treatment led to 17% ($P = 0.0168$) and 32% ($P = 0.1221$) reduction in SF_2, respectively (Fig. 5, Table 5). The corresponding decreases in SF_6 were 50% ($P = 0.0838$) and 67% ($P = 0.0364$). Combined treatment also reduced the mean inactivation dose by up to 18%, although this reduction did not reach statistical significance ($P \geq 0.2275$).

Exposure to the 1000-Hz modulated radiofrequency field, regardless of sequence, further sensitized L132 cells to X-rays (Fig. 5B, Table 5). SF_2, SF_6, and $\bar{D}$ were reduced by up to 62% ($0.0071 \leq P \leq 0.0086$), 92% ($0.0111 \leq P \leq 0.0127$), and 50% ($0.0170 \leq P \leq 0.0245$), respectively. The modifying factors obtained from these parameters are also summarized in Table 5. Based on SF_2, SF_6, and $\bar{D}$, combined treatment with the 1000-Hz modulated RFF was found to be correspondingly 1.9-, 4.5, and 1.6-fold more potent, respectively, in radiosensitizing the L132 cells than the 100-Hz modulated field.

The radiosensitization seen in the 100 Hz + X-rays and X-rays + 100 Hz treatments was also apparent in the increase in the α/β ratio from 4.00 ± 1.60 Gy (X-ray only) to

TABLE 4: Summary of radiobiological parameters for the Be11 cell line. SF_2 and SF_6 denote the surviving fraction at 2 and 6 Gy, respectively. α and β are the linear and quadratic coefficients of cell inactivation, respectively. $\bar{D}$ denotes the mean inactivation dose (area under the cell survival curve). Data are presented as the mean ± SEM from three independent experiments. Modifying factors (*MF*), relative to X-ray treatment alone, derived from the SF_2, SF_6, and $\bar{D}$ values according to Eq. (3)

Treatment	SF_2	SF_6	$\bar{D}$ (Gy)	α (Gy^{-1})	β (Gy^{-2})	MF_{SF2}	MF_{SF6}	$MF_{\bar{D}}$
X-rays	0.67 ± 0.05	0.08 ± 0.01	3.06 ± 0.03	0.09 ± 0.07	0.06 ± 0.01	—	—	—
100 Hz + X-rays	0.36 ± 0.04	0.02 ± 0.01	2.24 ± 0.28	0.44 ± 0.09	0.04 ± 0.01	1.86 ± 0.25	4.10 ± 1.42	1.37 ± 0.17
X-rays + 100 Hz	0.41 ± 0.11	0.03 ± 0.01	2.38 ± 0.34	0.40 ± 0.12	0.04 ± 0.01	1.63 ± 0.46	3.28 ± 1.55	1.29 ± 0.18
1000 Hz + X-rays	0.26 ± 0.04	0.01 ± 0.00*	1.67 ± 0.12	0.62 ± 0.08	0.02 ± 0.01	2.58 ± 0.44	6.31 ± 2.22	1.83 ± 0.13
X-rays + 1000 Hz	0.27 ± 0.03	0.01 ± 0.00*	1.77 ± 0.06	0.61 ± 0.07	0.02 ± 0.01	2.48 ± 0.33	6.83 ± 2.07	1.73 ± 0.06

*Errors less than 0.01; actual values were used for propagation of errors in modifying factors.

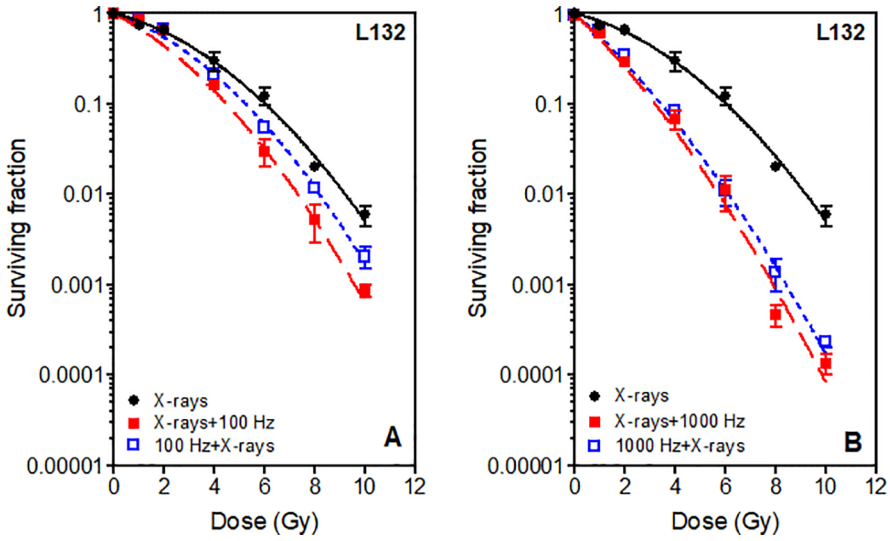

FIG. 5: Clonogenic survival curves for the L132 cell line after X-ray irradiation alone (solid circle, solid curve) and in combination with 100-Hz (A) and 1000-Hz (B) modulated radiofrequency fields (RFF). RFF exposure was performed 2 h prior to (open square, short dashed curve) or after (solid square, long dashed curve) X-ray treatment. The survival curves were obtained by fitting data from three independent experiments to the linear-quadratic model [Eq. (2)].

5.50 ± 0.92 and 8.25 ± 3.05 Gy, respectively. The more pronounced radiosensitization in the 1000 Hz + X-rays and X-rays + 1000 Hz treatments was reflected in even larger ratios of 18.67 ± 6.65 and 20.33 ± 7.28, respectively.

To obtain an overall indication of the relative radiosensitivity of the cell lines, a rank order was constructed based on SF_2, SF_6, and $\bar{D}$, as presented in Table 6. Except for combined treatment with the 1000-Hz modulated radiofrequency field, $\bar{D}$ emerged as the parameter providing the most consistent ranking of radiosensitivity. Using the frequency of cell lines under each rank for X-ray treatment alone, they may be arranged in order of increasing radiosensitivity as DU145 → L132 → Be11 → MeWo.

Radiosensitivity ranking for the combined treatment with the 100-Hz modulated RFF was similar to the intrinsic radiosensitivity ranking. The ranking obtained from the combined treatment with the 1000-Hz modulated RFF differed markedly from the intrinsic ranking, and emerged as DU145 → MeWo → L132 ≈ Be11, with the MeWo cells showing an increased treatment resistance.

IV. DISCUSSION

This study sought to compare intrinsic radiosensitivity to RFF modulated radiosensitivity, using the p53 mutant, human malignant melanoma MeWo cell line; the p53 wild-type, human melanoma Be11 cell line; the p53 mutant, human prostate cancer DU145 cell line; and the p53 wild-type, human normal lung epithelial L132 cell line. The intrinsic

TABLE 5: Summary of radiobiological parameters for the L132 cell line. SF_2 and SF_6 denote the surviving fraction at 2 and 6 Gy, respectively. α and β are the linear and quadratic coefficients of cell inactivation, respectively. $\bar{D}$ denotes the mean inactivation dose (area under the cell survival curve). Data are presented as the mean ± SEM from three independent experiments. Modifying factors (*MF*), relative to X-ray treatment alone, derived from the SF_2, SF_6, and $\bar{D}$ values according to Eq. (3)

Treatment	SF_2	SF_6	$\bar{D}$ (Gy)	α (Gy^{-1})	β (Gy^{-2})	MF_{SF2}	MF_{SF6}	$MF_{\bar{D}}$
X-rays	0.66 ± 0.07	0.12 ± 0.02	3.33 ± 0.42	0.16 ± 0.05	0.04 ± 0.01	—	—	—
100 Hz + X-rays	0.55 ± 0.02	0.06 ± 0.00*	2.90 ± 0.08	0.22 ± 0.03	0.04 ± 0.00*	1.20 ± 0.13	1.90 ± 0.40	1.15 ± 0.15
X-rays + 100 Hz	0.45 ± 0.08	0.04 ± 0.01	2.72 ± 0.11	0.33 ± 0.09	0.04 ± 0.01	1.47 ± 0.30	3.22 ± 1.12	1.22 ± 0.16
1000 Hz + X-rays	0.29 ± 0.04	0.01 ± 0.00*	1.82 ± 0.07	0.56 ± 0.07	0.03 ± 0.01	2.28 ± 0.40	9.67 ± 3.14	1.83 ± 0.24
X-rays + 1000 Hz	0.2 5± 0.04	0.01 ± 0.00*	1.67 ± 0.04	0.61 ± 0.08	0.03 ± 0.01	2.64 ± 0.51	12.89 ± 3.14	1.99 ± 0.26

*Errors less than 0.01; actual values were used for propagation of errors in modifying factors.

TABLE 6: Summary of relative radiosensitivity of DU145, L132, Be11, and MeWo cell lines based on SF_2, SF_6, and $\bar{D}$

Treatment	Parameter	Increasing Radiosensitivity →			
X-rays	SF_2	DU145	Be11	L132	MeWo
	SF_6	DU145	L132	Be11	MeWo
	$\bar{D}$	DU145	L132	Be11	MeWo
100 Hz + X-rays	SF_2	DU145	L132	MeWo	Be11
	SF_6	DU145	L132	MeWo	Be11
	$\bar{D}$	DU145	L132	Be11	MeWo
X-rays + 100 Hz	SF_2	DU145	L132	Be11	MeWo
	SF_6	DU145	L132	Be11	MeWo
	$\bar{D}$	DU145	L132	Be11	MeWo
1000 Hz + X-rays	SF_2	DU145	MeWo	L132	Be11
	SF_6	DU145	MeWo	L132	Be11
	$\bar{D}$	DU145	MeWo	L132	Be11
X-rays + 1000 Hz	SF_2	DU145	MeWo	Be11	L132
	SF_6	DU145	Be11	MeWo	L132
	$\bar{D}$	DU145	MeWo	Be11	L132

radiosensitivity data summarized in Table 6 show the DU145 to be the most radioresistant cell line and MeWo the most radiosensitive, giving a rank order of increasing radiosensitivity of DU145 → L132 → Be11 → MeWo, when the cell survival SF_2, SF_6, and $\bar{D}$ were collectively taken into account. Within the limits of experimental uncertainty, the SF_2 values obtained here are consistent with those reported previously for the DU145,[33,34] L132,[35] Be11,[36,37] and MeWo[36] cell lines, indicating no unusual radiation response.

As stated before, an appropriate radiomodulator is one that is nontoxic on its own. These data demonstrate that cellular exposure to radiofrequency fields, irrespective of frequency, does not have a notable cytotoxic effect, as the plating efficiency of the cell lines remained virtually unchanged. This is not surprising, as several other studies have not demonstrated cytotoxic effects in a variety of cellular systems even at extremely high radiofrequency fields.[38–40] As such, radiofrequency fields could, therefore, find application as efficient radiation modulators in radiotherapy or radiation protection.

In this investigation, combined treatment with the 100-Hz modulated RFF and X-rays does not markedly affect the radiosensitivity ranking of the cell lines (Table 6). Pre- and post-exposure to 100 Hz only slightly altered radiosensitivity, with DU145 and MeWo the most radioresistant and most radiosensitive cell lines, respectively, for the most part, the exception being when cells were pre-exposed to 100 Hz, which rendered Be11 the most radiosensitive cell line. Pre- and post-exposure to the 1000-Hz modulated RFF, however, augmented the radiosensitivity of the Be11 and L132 cell lines significantly more than the DU145 and MeWo cell lines, making Be11 and L132 more radiosensitive

than MeWo, with DU145 the most radioresistant in all treatments. The marked radiosensitization in the human lung fibroblasts (Table 5) contrasts the finding of the initial study, using Chinese hamster lung fibroblasts (V79), which showed that RFF can act as radioprotectors in normal cells.[22] This suggests that a potential application of the 1000-Hz modulated RFF as an adjuvant in the clinical setting might not be generally appropriate, as it may significantly aggravate radiation-induced normal tissue toxicity.

The disparity in radiomodulation by RFF seems to be partly influenced by the p53 status of the cell line. The p53 wild-type cell lines (L132 and Be11) were consistently more sensitized compared to the p53 mutant cell lines (MeWo and DU145), as is apparent from the modifying factors presented in Tables 2–5. The slight sensitization of the p53 mutant cell lines used here and the radioprotection demonstrated in the apparently normal V79 cells in a previous study[22] suggest that RFF influence radiosensitivity in ways that may be independent of p53 function. It is worth noting that p53 is also mutated and nonfunctional in V79 cells.[41] However, the significant sensitization of p53 wild-type cell lines suggests that RFF might target the p53 survival pathway, influencing it to enhance radiosensitivity. This would contrast with the report by Hirose and colleagues suggesting that radiofrequency fields in the GHz range do not affect p53 phosphorylation.[38] However, the effects of radiofrequency fields on macromolecules are largely frequency dependent.[42] The fields used in the current study may enhance radiation-induced damage to macromolecules, but damaged cells that are p53 mutant may evade p53-mediated apoptosis, dying through other modes[43] or surviving, while their p53 wild-type counterparts are eliminated through a p53-mediated apoptotic process. This further emphasizes the need for caution in possible combination of RFF with ionizing radiation in cancer therapy, as a significant component of cancers are p53 mutated and such mutations might sometimes infer gain of certain protective functions.[44] It is also possible that RFF exposure activates other genes in different survival pathways, the influence on survival depending on whether genes in the targeted pathway are functional or altered, rendering them dysfunctional. The presence of a dysfunctional gene in a survival pathway brings discontinuity to a pathway.

The influence of RFF seems to be cell line and frequency dependent,[22] more so at higher radiation doses. The radiofrequency fields evaluated here affect the radiosensitivity to more or less the same extent. These results suggest that any potential use of RFF, as an adjuvant to radiation therapy, needs to be regulated and guided by the characteristics of each cancer, e.g., type and p53 status. This, especially that RFF modulated at higher frequencies (2000 and 4000 Hz) tended to promote cell survival at the higher radiation dose of 6 Gy (data not shown). Such radioprotection by fields modulated at high frequency could have significant implications for the potential use of RFF in hypofractionation settings where large fractional doses are employed.

V. CONCLUSIONS

The data presented here show that radiofrequency fields are more efficient in modulating large fractional doses of X-rays and could find application in hypofractionated radiotherapy

as adjuvants, especially for tumors with low alpha/beta ratios. Radiofrequency fields modulate cellular radiosensitivity in a frequency- and cell-type-dependent manner. Their effects on radiosensitivity also appear to be linked to p53 status, with cells with mutant p53 being less sensitized than their p53 wild-type counterparts. These findings can have a significant positive impact on the management of patients with superficial tumors that may be resistant to low fractional doses of radiation, and specifically tumors that are p53 wild-type.

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