Radiofrequency Field–Induced Radiosensitization Is Related to Reductions in Metabolic Activity

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ABSTRACT: Although radiofrequency fields (RFFs) have been found to exhibit both radiosensitizing (enhancement of radiation) and radioprotective (mitigation of radiation) effects, mechanisms underlying these phenomena have not been clearly elucidated. Here, we use four human cell lines, namely, MeWo and Be11 (melanomas), DU145 (prostate carcinoma), and L132 (normal lung fibroblasts), to assess the role of RFF modulation of cellular metabolic activity in altering radiosensitivity. We measure radiosensitivity and metabolic activity using colony-forming and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays, respectively. Cell lines that are more radiosensitized by RFF exposure show larger reductions in metabolic activity, relative to radiation treatment alone, regardless of whether RFF exposure occurs before or after X-ray irradiation. The finding that surviving cells maintain elevated metabolic activity when treated with a combination of RFFs and X-rays suggests that changes in metabolic activity may be triggered by RFFs to support processes such as DNA repair and alteration of long-term cell survival. Modulation of cellular metabolic activity by RFFs may have important ramifications for moderating ionizing radiation–induced effects. This must be carefully considered if RFFs are to be applied as adjuvants in radiotherapy.

KEY WORDS: radiofrequency fields, metabolic activity, adjuvant radiotherapy

I. INTRODUCTION

Although the mechanisms by which electromagnetic fields interact with cells are not well understood, many studies have shown that electromagnetic fields with a wide range of frequencies can influence multiple cellular processes, such as proliferation, differentiation, cycle, apoptosis, DNA replication, production of reactive oxygen species, and protein/gene expression.1–9 Effects of electromagnetic fields extend to their capacity to stimulate the immune system,10 which may in turn mediate cellular responses to therapeutic interventions. Additionally, it has been suggested that some electromagnetic fields, especially those at high frequencies, can disrupt nervous system function and result in neurodegenerative disorders including autism,11 whereas others at lower frequencies stimulate damaged tissue recovery.12–17

Given that radiofrequency waves show potential to act as radiosensitizers (enhancers of radiation effects) and radioprotectors (mitigators of radiation effects),18,19 we must understand the mechanisms underlying their modulatory effects. This may assist in
designing patient- and tumor-specific therapeutic approaches that lead to more effective cancer management.

Using human melanoma, prostate cancer cells, and normal lung fibroblasts, we assess the effect of radiofrequency field (RFF) exposure on ionizing radiation-induced changes in cellular metabolic activity. We also discuss the potential link between RFF-induced changes in radiosensitivity and radiation-induced alterations in metabolic activity.

II. MATERIALS AND METHODS

A. Cell Lines and Culture Maintenance

In this study, we used four human cell lines, namely, MeWo and Be11 (melanomas), DU145 (prostate carcinoma), and L132 (normal lung fibroblasts). Cell cultures were maintained as described in an investigation by Chinhengo et al. For clonogenic cell survival and metabolic assays, exponentially growing cells were trypsinized into single-cell suspensions and seeded appropriately.

B. Clonogenic Survival

We used cell survival data from a study by Chinhengo et al. to evaluate the relationship between radiosensitivity modulation by 100- and 1000-Hz–amplitude modulated RFFs and changes in metabolic activity.

C. Assessment of Metabolic Activity

We measured treatment-induced changes in cellular metabolic activity using a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. For this, 100,000 cells in 100 µL of medium were seeded per well into a 96-well cell culture plate (four wells per sample), with detachable wells enabling selective treatment and reassembly. Cells were incubated for 2 h to attach. Sets of wells were then used for each treatment option of radiofrequency waves modulated by either 100 or 1000 Hz for 2 h before and after 6-Gy irradiation with X-rays. Wells treated with RFFs and X-rays alone were used as negative and positive controls, respectively. After treatment, wells were reassembled and cultures were incubated for 30 min (to measure early changes in metabolic activity) and 18 h (to measure late changes in metabolic activity) at 37°C in a humidified atmosphere (95% air, 5% CO₂).

After each incubation period, 10 µL of 5-mg/mL MTT solution (prepared by dissolving MTT powder in phosphate-buffered saline) were added to each well and incubated for 4 h in the dark at 37°C in a humidified atmosphere (95% air, 5% CO₂). During this period, metabolically active cells reduced MTT to generate purple formazan crystals. We added 100 µL dimethyl sulfoxide to each well to solubilize the crystals, and the plate was incubated for 10 min at room temperature in the dark. We determined absorbance (measured as optical density [OD]) for samples and blanks at a wavelength
of 560 nm, using a microplate spectrophotometer (Labtech International, Sussex, UK; model no. LT-4000).

Data were expressed as relative metabolic activity, given as ratios of mean absorbance in samples treated with X-rays to those obtained for negative controls (OD\textsubscript{6Gy}/OD\textsubscript{0Gy}) or ratios of mean absorbance in triplicate samples treated with combinations of X-rays and RFFs to those obtained for positive controls (OD\textsubscript{6Gy-RFF}/OD\textsubscript{0Gy-RFF}). Ratios of the former to the latter (or modifying factors) were then used to represent the mode by which RFFs modified metabolic activity in irradiated cells. The subscript 6Gy+RFF denotes RFF exposure after X-ray irradiation. A modifying factor of > 1, 1, or < 1 indicates a reduction, no effect, or an enhancement in metabolic activity in irradiated cells by RFFs, respectively.

D. Statistical Analysis

Data analyses were performed with GraphPad Prism software (San Diego, CA). All data were presented as mean (± standard error of the mean) from three independent experiments. Errors for derived parameters were determined by using appropriate error propagation formulae. For associations, we used linear regression analyses.

III. RESULTS

A. Modulation of Radiosensitivity by RFFs

The effect of 100- and 1000-Hz–modulated RFF on radiosensitivity was expressed as a modifying factor \(MF\), given as the ratio of surviving fractions at 6 Gy in the absence and presence of RFFs.\cite{20} Modifying factors for the four cell lines are presented in Table 1. The criteria for radiosensitivity inhibition, no effect, and enhancement by RFFs are indicated by modifying factors < 1.0, 1.0, and > 1.0, respectively.

B. Effect of RFFs on Radiation-Induced Metabolic Changes

The MTT assay was used to assess the potential effect of RFF on early (30 min) and late (18 h) radiation-induced changes in metabolic activity of the four cell lines. We

<table>
<thead>
<tr>
<th>Treatment*</th>
<th>DU145</th>
<th>MeWo</th>
<th>Be11</th>
<th>L132</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 Hz + 6 Gy</td>
<td>1.15 ± 0.12</td>
<td>1.00 ± 0.56</td>
<td>4.10 ± 1.42</td>
<td>1.90 ± 0.40</td>
</tr>
<tr>
<td>6 Gy + 100 Hz</td>
<td>1.36 ± 0.15</td>
<td>1.16 ± 0.71</td>
<td>3.28 ± 1.55</td>
<td>3.22 ± 1.12</td>
</tr>
<tr>
<td>1000 Hz + 6 Gy</td>
<td>1.50 ± 0.32</td>
<td>2.42 ± 0.32</td>
<td>6.31 ± 2.22</td>
<td>9.67 ± 3.14</td>
</tr>
<tr>
<td>6 Gy + 1000 Hz</td>
<td>1.67 ± 0.39</td>
<td>3.22 ± 0.49</td>
<td>6.83 ± 2.07</td>
<td>12.89 ± 3.14</td>
</tr>
</tbody>
</table>

*100 Hz + 6 Gy and 6 Gy + 100 Hz denote radiofrequency exposure before and after X-ray irradiation, respectively. Errors were calculated by using error propagation formulae for ratios.
further evaluated whether such changes have a role in long-term cell survival. For this, treatment-induced changes in metabolic activity were expressed as fold changes (relative metabolic activity) relative to appropriate controls (see section IIIC, below).

Figure 1 shows fold changes in metabolic activity in the prostate cancer cell line DU145 for the various treatments. Whereas treatment with 6 Gy or 6 Gy + 1000-Hz modulated RFFs (X-ray irradiation followed by RFF exposure) resulted in a reduction in early metabolic activity by ~60%, a 6 Gy + 100-Hz modulated RFF treatment yielded

**FIG. 1:** Relative metabolic activities for prostate cancer cell line DU145: (A) 30 min and (B) 18 h after treatment with 6 Gy of X-rays, alone or in combination with 100- and 1000-Hz modulated RFFs. For instance, 100 Hz + 6 Gy and 6 Gy + 100 Hz denote radiofrequency exposure before and after X-ray irradiation, respectively.
an enhancement (~1.3-fold) in metabolic activity (Fig. 1A). No effect on early metabolic activity was apparent when cells were exposed to RFF before X-ray treatment.

Similarly, no effect on late metabolic activity was observed when cells were treated with either X-rays alone or X-rays followed by a 100-Hz modulated RFF (Fig. 1B). However, exposure to a 100-Hz modulated field followed by X-ray treatment or X-ray treatment followed by exposure to a 1000-Hz modulated RFF resulted in increased (~1.3-fold) late metabolic activity. In contrast to the absence of an effect at an early time point, pre-exposure to the 1000-Hz modulated field followed by X-ray treatment led to a reduction of ~32% in late metabolic activity (Fig. 1B).

Determination of dose-modifying factors, as described in Section 3C, revealed that regardless of RFF frequency and sequence of combination with X-rays, early metabolic activity was enhanced ($MF < 1.0$; Table 2). Late metabolic activity also increased in 100-Hz modulated RFF + X-ray and X-ray + 1000-Hz modulated RFF treatments, whereas a reduction ($MF > 1.0$; Table 2) occurred in the 1000-Hz modulated RFF + X-ray treatment. Exposing cells to the 100-Hz modulated RFF after irradiation did not affect late metabolic activity.

**TABLE 2: MF, relative to X-ray treatment alone, derived from the relative metabolic activities presented in Figs. 1–4 for DU145, MeWo, Be11, and L132 cell lines, as described in section IIIC of the text.**

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Treatment$^a$</th>
<th>30 min</th>
<th>18 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>DU145</td>
<td>100 Hz + 6 Gy</td>
<td>0.39 ± 0.09</td>
<td>0.75 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>6 Gy + 100 Hz</td>
<td>0.32 ± 0.04</td>
<td>0.99 ± 0.38</td>
</tr>
<tr>
<td></td>
<td>1000 Hz + 6 Gy</td>
<td>0.40 ± 0.09</td>
<td>1.42 ± 0.29</td>
</tr>
<tr>
<td></td>
<td>6 Gy + 1000 Hz</td>
<td>0.89 ± 0.22</td>
<td>0.69 ± 0.15</td>
</tr>
<tr>
<td>MeWo</td>
<td>100 Hz + 6 Gy</td>
<td>0.85 ± 0.22</td>
<td>1.05 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>6 Gy + 100 Hz</td>
<td>1.09 ± 0.33</td>
<td>1.27 ± 0.36</td>
</tr>
<tr>
<td></td>
<td>1000 Hz + 6 Gy</td>
<td>0.73 ± 0.09</td>
<td>0.98 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>6 Gy + 1000 Hz</td>
<td>1.37 ± 0.20</td>
<td>1.08 ± 0.20</td>
</tr>
<tr>
<td>Be11</td>
<td>100 Hz + 6 Gy</td>
<td>0.28 ± 0.09</td>
<td>2.27 ± 0.60</td>
</tr>
<tr>
<td></td>
<td>6 Gy + 100 Hz</td>
<td>0.35 ± 0.04</td>
<td>2.04 ± 0.34</td>
</tr>
<tr>
<td></td>
<td>1000 Hz + 6 Gy</td>
<td>0.65 ± 0.13</td>
<td>1.91 ± 0.21</td>
</tr>
<tr>
<td></td>
<td>6 Gy + 1000 Hz</td>
<td>0.55 ± 0.12</td>
<td>1.56 ± 0.16</td>
</tr>
<tr>
<td>L132</td>
<td>100 Hz + 6 Gy</td>
<td>2.44 ± 0.82</td>
<td>0.52 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>6 Gy + 100 Hz</td>
<td>1.85 ± 0.54</td>
<td>0.83 ± 0.30</td>
</tr>
<tr>
<td></td>
<td>1000 Hz + 6 Gy</td>
<td>4.19 ± 1.18</td>
<td>1.47 ± 0.49</td>
</tr>
<tr>
<td></td>
<td>6 Gy + 1000 Hz</td>
<td>2.55 ± 0.75</td>
<td>2.00 ± 0.71</td>
</tr>
</tbody>
</table>

$^a$100 Hz + 6 Gy and 6 Gy + 100 Hz denote radiofrequency exposure before and after X-ray irradiation, respectively. Errors were calculated by using error propagation formulae for ratios.
Data in Fig. 2 show changes in metabolic activity in the melanoma cell line MeWo following different treatments. Except for the X-ray + 1000-Hz modulated RFF treatment that resulted in a reduction, the other treatments either incurred no effect or induced an increase in early metabolic activity (Fig. 2A). With experimental uncertainty, all treatments had no effect on late metabolic activity (Fig. 2B).

FIG. 2: Relative metabolic activities for the melanoma cell line MeWo: (A) 30 min and (B) 18 h after treatment with 6 Gy of X-rays alone or in combination with 100- and 1000-Hz modulated RFFs. For instance, 100 Hz + 6 Gy and 6 Gy + 100 Hz denote radiofrequency exposure before and after X-ray irradiation, respectively.

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Dose-modifying factors presented in Table 2 demonstrate no marked changes in metabolic activity in the MeWo cells, except for 100- and 1000-Hz modulated RFF + X-ray treatments that resulted in ~1.2- and 1.4-fold enhancements in early metabolic activity, respectively, when compared with those of X-ray–only treatment.

X-ray treatment alone resulted in an ~50% reduction and a similar extent of enhancement in early and late metabolic activity in the Be11 cell line (Fig. 3). Combined X-ray and RFF treatments of these cells, regardless of sequence, enhanced early metabolic

FIG. 3: Relative metabolic activities for the melanoma cell line Be11: (A) 30 min and (B) 18 h after treatment with 6 Gy of X-rays alone or in combination with 100- and 1000-Hz modulated RFFs. For instance, 100 Hz + 6 Gy and 6 Gy + 100 Hz denote radiofrequency exposure before and after X-ray irradiation, respectively.
activity (Fig. 3A; Table 2). On the other hand, all combination treatments with both RFFs led to a reduction in late metabolic activity (Fig. 3B; Table 2).

Relative metabolic activity data presented in Fig. 4 show that X-ray irradiation of the normal lung fibroblasts L132 resulted in an ~60% increase in early metabolic activity (Fig. 4A). Inclusion of RFF exposure produced either no effect (6 Gy + 100-Hz modulated RFF) or reduced (all other combined treatments) metabolic activity. On the other hand, a 6-Gy exposure alone resulted in an ~40% reduction in late metabolic activity.

**FIG. 4:** Relative metabolic activities for human lung fibroblasts L132: (A) 30 min and (B) 18 h after treatment with 6 Gy of X-rays alone or in combination with 100- and 1000-Hz modulated RFFs. For instance, 100 Hz + 6 Gy and 6 Gy + 100 Hz denote radiofrequency exposure before and after X-ray irradiation, respectively.
activity (Fig. 4B). The 100-Hz modulated RFF + X-ray treatment enhanced late metabolic activity. However, the other combined treatments resulted in reduced activity, with 1000-Hz modulated RFF treatment most effective (Fig. 4B).

Dose-modifying factors for lung fibroblasts in Table 2 show that combined X-ray and RFF treatment, regardless of sequence, led to a reduction ($MF > 1.0$) in early metabolic activity. A similar reduction in late metabolic activity also occurred when cells were concomitantly treated with 1000-Hz modulated RFF. However, combined treatment with 100-Hz modulated RFF increased ($MF < 1.0$) late metabolic activity.

C. Relationship between Radiosensitivity and Metabolic Activity

To evaluate whether treatment-induced changes in metabolic activity have a role in the modulatory effects that RFFs have on cellular radiosensitivity, modifying factors that were derived from the MTT assay were plotted as a function of those obtained from the clonogenic cell survival assay. Data in Fig. 5 show a weak correlation ($Y = [0.14 \pm 0.06] X + [0.20 \pm 0.39]$; $R^2 = 0.4767$; $p = 0.058$) between the 100-Hz RFF induced radiosensitization and changes in early metabolic activity (Fig. 5A), where cell lines displaying high levels of radiosensitization on the basis of clonogenic survival tended to have reduced ($MF > 1.0$) metabolic activity. No relationship was apparent between early metabolic activity and cell survival when 1000-Hz modulated RFF was used.

No link emerged between cell survival and late changes in metabolic activity for combined treatment with the 100-Hz modulated RFF (Fig. 6A). However, when only malignant cell lines were considered, a strong correlation became apparent, wherein an increase in radiosensitization was mirrored by a reduction in metabolic activity. Similarly, a correlation ($Y = [0.08 \pm 0.03] X + [0.92 \pm 0.20]$; $R^2 = 0.5851$; $p = 0.027$) emerged between changes in late metabolic activity and radiosensitization by the 1000-Hz modulated RFF (Fig. 6B).

IV. DISCUSSION

To determine the effect of RFFs on X-ray induced changes in metabolic activity, relative metabolic activities that were determined at 30 min and 18 h were used to derive modifying factors, as described in section IIIC. We expected that the more radiosensitized cell lines would exhibit relatively higher metabolic activity (increased metabolic activity, represented here by $MF_{MTT} < 1.0$) and vice versa, because evidence suggests that reduced metabolic rates lead to radioresistance.21-23 However, data in Fig. 5 (especially for the 100-Hz modulated RFF) seem to reflect the opposite, with a trend toward cell lines that were more radiosensitized by RFF exposure showing larger reductions in metabolic activity in relation to radiation treatment alone, regardless of treatment sequence. No link was found between intrinsic radiosensitivity and metabolic rate in untreated cell cultures, suggesting that the observed trend may be due to treatment-related alterations in metabolic rate. Within the first hour of treatment, changes in metabolic activity might trigger support processes such as DNA repair.
FIG. 5: Plot of modifying factors from metabolic activity (measured 30 min after treatment) as a function of modifying factors from clonogenic cell survival for four cell lines. Combined treatment with: (A) 100-Hz modulated RFF and (B) 1000-Hz modulated RFF. Metabolic activity-modifying factor $MF_{MTT}$ is the ratio of fold change in metabolic activities in cultures treated with a combination of X-rays and RFFs to those treated with X-rays alone. A modifying factor of > 1, 1, or < 1 indicates a reduction, no effect, or an enhancement in metabolic activity in irradiated cells by RFFs, respectively. The survival modifying factor ($MF_{survival}$) is the ratio of surviving fractions at 6 Gy of X-rays in the absence of RFF exposure to those in the presence of RFF exposure. Criteria for inhibition, no effect, and enhancement of radiosensitivity by RFFs are $MF_{survival} < 1.0$, $MF_{survival} = 1.0$, and $MF_{survival} > 1.0$, respectively. Dashed lines represent a 95% confidence interval. MF, modifying factor.
FIG. 6: Plot of modifying factors from metabolic activity (measured 18 h after treatment) as a function of modifying factors from clonogenic cell survival for four cell lines. Combined treatment with: (A) 100-Hz modulated RFF and (B) 1000-Hz modulated RFF. Metabolic activity modifying factor $MF_{MTT}$ is the ratio of fold change in metabolic activities in cultures treated with a combination of X-rays and RFFs to those treated with X-rays alone. A modifying factor of $> 1$, $1$, or $< 1$ indicates reduction, no effect, or enhancement in metabolic activity in irradiated cells by RFFs, respectively. The survival modifying factor $MF_{survival}$ is the ratio of surviving fractions at 6 Gy of X-rays in the absence of RFF exposure to those in the presence of RFF exposure. Criteria for inhibition, no effect, and enhancement of radiosensitivity by RFFs are $MF_{survival} < 1.0$, $MF_{survival} = 1.0$, and $MF_{survival} > 1.0$, respectively. Dashed lines represent a 95% confidence interval. MF, modifying factor.
This relationship between metabolic rate and radiosensitization persisted, especially for the cancer cell lines, when metabolic activity was assessed at a much later time point (Fig. 6). At 18 h post-treatment, increased cellular metabolism may be thought to support processes such as proliferation, whereas reduction might signal cell cycle arrest. The phenomenon of RFF-induced increases in metabolic activity may be the result of electromagnetic fields causing cells to move from inactive cell cycle phases to the more active G2/M phases, with associated elevations in proliferation.24,25 Enhanced proliferation could render cells vulnerable to radiation damage and lead to high levels of radiosensitization. However, effects elicited by electromagnetic fields seem to be quite dependent on cell type. Although high-frequency RFFs have been shown to induce significant levels of cell cycle arrest in neuronal cells,26 no such effects were demonstrated in fibroblasts and glioma cells.27 Such differential influences of electromagnetic fields can lead to radiosensitization, radioprotection, or no effect on cellular radiosensitivity.

Our results suggest that metabolic activity may be a determinant of long-term survival. Assessment of how RFFs affect radiation-induced cell killing in an expanded panel of cell lines could clarify the importance of p53 status in radiomodulation by RFFs.

V. CONCLUSIONS

Here, we demonstrated that metabolic activity in cell lines that are relatively more radiosensitized by RFF exposure is reduced by a larger extent, relative to radiation treatment alone, regardless of the sequence in which X-rays and RFF are administered. This finding could have important implications in the manner by which RFFs moderate ionizing radiation-induced effects and must be considered in potential applications of RFFs as adjuvants to radiotherapy.

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