Differential Radiosensitivity of Uveal Melanoma Cell Lines After X-rays or Carbon Ions Radiation

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PURPOSE. We compared the radiosensitivity of uveal melanoma (UM) cell lines after x-ray or carbon-ion radiation (C-ions).

METHODS. We characterized the radiosensitivity toward x-rays and C-ions of UM cell lines: 92.1, MEL270, SP6.5, MTK-FBR, µ2, and TP17. Normal choroidal melanocytes and the retinal pigment epithelial cell line ARPE19 were used as controls for normal cells. X-rays were delivered with an energy of 6 MV at a dose rate of 2 Gy/min. X-rays served as a reference for Relative Biological Effectiveness (RBE) evaluation. Radiation with C-ions was delivered at 75 MeV/u (34 keV/µm) at a dose rate of 2 Gy/min. After single-doses (0–8 Gy) of medical x-rays (6 MV) or C-ions (33 keV/µm), cells sensitivity was measured using standard colony formation assay, and cell growth was examined by counting the cell colonies. The effect of x-rays or C-ions on the expression and activation of ERK1/2 was evaluated by Western Blot.

RESULTS. C-ions presented with regard to the x-rays a RBE of 1.9 to 2.5 at 10% of UM cells survival. The x-ray sensitivity of UM cells was neither influenced by the synchronization of cells in phase G0/G1 of the cell cycle nor by the level of oxygenation. X-ray and C-ions radiation had the same effects on cell cycle leading to a mitotic catastrophe that appeared earlier after C-ions than x-ray treatment. However, C-ions radiation induced a sustained inhibition of ERK1/2 activation compared to the transitory induction of that signalization pathway after x-ray radiation.

CONCLUSIONS. This in vitro study shows that C-ions had a better biological effectiveness than x-rays leading to a sustained inhibition of the ERK1/2 pathway.

Keywords: x-rays, carbon-ion radiation, hadron therapy, uveal melanocyte, uveal melanoma, ERK1/2 activation

Uveal melanoma (UM) is the most common primary ocular malignancy in adults.1 The first choice of conservative treatment for UM patients is plaque brachytherapy, delivered by plaque-shaped applicators containing isotopes, such as iodine-125 or ruthenium-106, that emit electrons, x-rays, and, respectively, γ and β rays.2 Another treatment modality is charged particle radiation therapy (hadron therapy) delivered with either protons or carbon ions.3–5 Hadron therapies are characterized by a high linear energy transfer (LET), which means that they give up a lot of energy in a small volume, therefore producing less damages to the surrounding tissues than x-rays. The more widely used mode of charged particle therapy is protontherapy.5,6 Another recent modality of hadron therapy uses carbon ions (C-ions). Because C-ions are heavier than protons, their relative biological efficiency (RBE) is consequently expected to be superior.7 The RBE for C-ions is the ratio of the dose of C-ions and the dose of standard x-rays required to produce the same biological effect.7 Therefore, C-ions could offer an effective treatment alternative that may achieve comparable tumor control probability with a lower physical dose than that used in proton therapy, especially for radioresistant tumors, such as chondrosarcomas, chordomas, and adenoid cystic carcinomas.7,8 Moreover, hypoxia can compromise the beneficial effects of ionizing radiation by the production of organic peroxide that can induce permanent damage to DNA.9 Under hypoxic conditions however, DNA damage induced by low linear energy transfer radiation (x-rays) can be more readily repaired while the direct action caused by high linear energy transfer radiation (C-ions) is less affected by the presence of oxygen.10,11 Indeed, more than 3000 patients have been treated with C-ions worldwide, including UM patients.5,7,12
of Radiologic Sciences in Japan that comprises 55 patients with UM has proven the feasibility and safety of C-ions radiation for UM treatment, but the radiosensitivity of melanoma requires further studies, especially for C-ions, to improve the therapeutic outcome.18,19

The most important deregulation observed at the cellular level in more than 86% of UM is the constitutive activation of the ERK1/2 pathway. Our previous findings showed the key role of the WTBRaf/MEK/ERK pathway in the control of UM cells proliferation.20 To date, exclusive somatic mutations in the heterotrimeric G protein-encoding genes GNAQ and GNA11 were observed in 46% and 32% of UM, respectively.15–17 These mutations were reported to cause activation of ERK1/2 in UM. Previous studies have found that oncogenic RAS and RAF, reported to cause the activation of ERK1/2, also enhance radiation resistance in a variety of unrelated human tumor cell types.18,19 We have shown previously that UM cell lines display constitutively high levels of activated ERK1/2 that, in some cases, may be explained by the expression of either B-Raf, or mutations in either GNAQ or GNA11.14,20 Several studies have reported that activating mutations activate ERK1/2, which in turn results in the activation of several transcription factors regulating genes involved in cell growth.22–25 Finally, constitutive activation of ERK may diminish the lethal effects of radiation in normal human and tumor cells.26

In this study, the radiosensitivity of UM cell lines toward x-ray and C-ions radiation was evaluated. Expression and activation of ERK1/2 also were compared after x-ray and C-ions radiation.

**Materials and Methods**

This study followed the tenets of the Declaration of Helsinki.

**Cell Culture**

Human UM cell lines 92.1 and MEL270 were provided by Martine Jager (Leiden University Medical Center, Leiden, The Netherlands). The human UM cell lines TP17 and SP6.5 cells were provided by the Quebec Uveal Melanoma Infrastructure from the Réseau de Recherche en Santé de la Vision of the FRQS. The human UM cell line µ2 was provided by Loris G. Baggetto (Lyon, France). The human MTK-BR was provided by José Sahel.27 The SP6.5 and MKT-BR cell lines exhibit the mutation V600E RAF, whereas MEL270 and µ2 cell lines display the mutation GNAQ/Q209P.14,28 These cells were cultured as described previously.21,29,30 Normal chorioidal melanocytes (NCM) were isolated from human enucleated eyes provided by the Faculty of Medicine of Caen, France and were cultured as described previously.20,29,30 The NCM-A cell line was provided by Leonard Hjelmeland, (University of California, Los Angeles, CA, USA) and was cultured as described previously.30 These cells were maintained at 37°C in a humidified incubator containing 5% CO2 and 95% air (referred to as normoxic conditions).

**Irradiation**

X-rays were delivered by a medical linear accelerator (Saturne 15; General Electric, Buc, Centre de Lutte contre le Cancer François Baclesse, Caen, France) with an energy of 6 MV at a dose rate of 2 Gy/min. X-rays served as a reference for RBE evaluation. Radiation with C-ions was performed on D1 line of GANIL accelerator (Caen, France). C-ions were delivered at 75 MeV/nu (3.4 keV/μm) at a dose rate of 2 Gy/min. Cells were subjected to single radiation doses of 1, 2, 3, 4, 6, and 8 Gy at room temperature. Cell survival was assessed by clonogenic assays. Experiments were conducted on exponentially growing and confluent cells. For the exponential growing phase, cells were seeded 12 to 16 hours before radiation. For confluent cells, they were seeded subconfluent 5 days before radiation to obtain G0/G1 phase-arrested cells (checked by flow cytometry). Cells were plated in adequate numbers 4 to 6 hours after irradiation to obtain 5 to 200 clones per flask at 10 days.

**Hypoxia Procedure**

Hypoxia experiments were performed in a hypoxic chamber (Ruskinn, InvivoO2 1000; Ruskinn Technology and Ave International, Blain, France) at 1% O2 and 5% CO2 at an appropriate N2 pressure to reach 100% gas at 37°C. The culture medium was replaced by the same medium equilibrated for 30 min with the gas mixture contained in the hypoxic chamber. Cells were maintained under hypoxic conditions for 5 to 6 hours after seeding and at least 12 hours before irradiation and then incubated in hypoxic conditions for 10 to 15 days.

**Cell Proliferation and Cell Staining**

To examine the cell’s ability to grow under normoxic and hypoxic conditions, cells were seeded at a density of 5 × 103 cells/well in 12-well plates and cultured for 6 days. Cells were counted each day using a cell counting plate and were counted and considered as cells with unaffected clonogenic capacity. Each individual experiment (n = 3) was performed on distinct days in triplicates for each condition. Survival (S) data after dose (D) of radiation were corrected for the plating efficiency (PE). The PE values correspond to the surviving fractions after 10 days of culture without radiation. The radiation survival determined from clonogenicity assays was fitted according to the linear-quadratic formula S(D) = exp(−αD−βD2). The value of α is related to the lethal damage, whereas β is related to the sublethal and potentially lethal damage that the cell could repair depending on its capacity and environment. This model was used to determine the percentage of cells surviving at 2 Gy (SF2) of x-ray and C-ions radiation. Doses of radiation required to reduce the surviving fraction to 37%, 10%, 1% (D37, D10, and D1, respectively) were used. The RBE of C-ions at D37 and D10 were calculated for each cell line using the following formula: RBE = D37 (37 x-rays/ D37 carbon ions and RBE D10 = D10 x-rays/ D 10 carbon ions.

**Western Blot Analysis**

Cells were harvested in a lysis solution containing 50 mM Tris-HCl (pH 7.6), 1% Nonidet P-40 (Sigma Aldrich), 150 mM NaCl, 1 mM phenylmethylsulfonyl fluoride and 2 mM EDTA in the presence of a protease inhibitor cocktail (1:100, Sigma Aldrich). Supernatant was isolated from centrifuged cell
The SF2 is the Surviving Fraction after 10 days of culture without irradiation. The PE is the surviving fraction after an irradiation of 2 Gray, $\alpha$ is the linear factor, and $\beta$ the quadratic factor. The ratio $\alpha/\beta$ is the irradiation dose corresponding to an equal contribution of the linear factor $\alpha$ and the quadratic factor $\beta$.

**Statistical Analysis**

Values are given as mean $\pm$ SEM. Data obtained in the various conditions were compared using unpaired $t$-tests. For all comparisons, values of $P < 0.05$ were considered significant. The statistical analysis was performed using the software Prism5 Graphpad (Graphpad, La Jolla, CA, USA).

**RESULTS**

**Cellular Radiosensitivity After X-rays**

The cellular surviving fractions of the UM cell lines, and the NCM and ARPE19 cells (used as controls) were plotted on a cell survival curve using the linear quadratic model. The PE ranged from 13% to 30% for UM cell lines and was lower for primary cultures of NCM (Table 1). The survival curve of the UM cell lines showed a large initial shoulder and an exponential decline in growth following the two-hits target linear quadratic model (Fig. 1). In contrast, NCM followed the one-hit target linear quadratic model ($\beta = 0$). The values of $\alpha$-component for all UM cell lines ($\alpha = 0.159–0.349$) were significantly lower than those of nontumoral cells ($\alpha_{\text{NCM}} = 0.488$ and $\alpha_{\text{ARPE19}} = 0.672$; Table 1).

**Factors Inducing X-ray Resistance**

We next used, among the six UM cell lines, the SP6.5 and MKT-BR cell lines that were the most x-ray sensitive and x-ray resistant, respectively. To assess the role of cell cycle progression in the mechanism of radiosensitivity, we compared the cell survival curves of asynchronous and confluent cells ($G_0/G_1$ cell cycle phase synchronization) after x-ray exposure for the SP6.5 and MKT-BR cell lines, and NCM (Fig. 2A). These three cell types showed a similar x-ray sensitivity in $G_0/G_1$ cell cycle phase synchronization compared to cells in exponential phase; therefore, suggesting that cycle progression has no influence on the cell survival after x-ray radiation.

To study the oxygen effect on melanoma cells, we next compared the SP6.5 and MKT-BR proliferation rates in normoxic (21% of O$_2$) and hypoxic (1% of O$_2$) conditions (Fig. 2B). When cultured under normoxic condition, SP6.5 and MKT-BR grew rapidly with a doubling time of approximately 28 to 30 hours (Fig. 2B). In contrast, these melanoma cells grew slowly, with a doubling time of approximately 44 to 48 hours under hypoxic condition. These data suggested that the decrease in the oxygen concentration also reduces the

**Table 1.** Cell Survival Parameters for NCM, ARPE 19, and Uveal Melanoma Cell Lines Cultured in Normoxia After Single Doses of X-rays

<table>
<thead>
<tr>
<th>Type of Irradiation</th>
<th>NCM</th>
<th>ARPE19</th>
<th>SP6.5</th>
<th>MEL270</th>
<th>92.1</th>
<th>MKT-BR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>X</td>
<td>C</td>
<td>X</td>
<td>C</td>
<td>X</td>
<td>C</td>
</tr>
<tr>
<td>SF2</td>
<td>0.259</td>
<td>0.226</td>
<td>0.362</td>
<td>0.148</td>
<td>0.448</td>
<td>0.140</td>
</tr>
<tr>
<td>$\alpha$, Gy$^{-1}$</td>
<td>0.672</td>
<td>0.860</td>
<td>0.488</td>
<td>1.149</td>
<td>0.349</td>
<td>1.004</td>
</tr>
<tr>
<td>$\beta$, Gy$^{-1}$</td>
<td>0</td>
<td>0</td>
<td>0.029</td>
<td>0</td>
<td>0.030</td>
<td>0</td>
</tr>
<tr>
<td>$\alpha/\beta$</td>
<td>N/A</td>
<td>N/A</td>
<td>16.83</td>
<td>N/A</td>
<td>11.63</td>
<td>N/A</td>
</tr>
<tr>
<td>PE, %</td>
<td>6</td>
<td>5</td>
<td>30</td>
<td>62</td>
<td>28</td>
<td>29</td>
</tr>
<tr>
<td>D37</td>
<td>1.3</td>
<td>0.9</td>
<td>3.8</td>
<td>0.4</td>
<td>3.3</td>
<td>0.9</td>
</tr>
<tr>
<td>D10</td>
<td>4.8</td>
<td>2.8</td>
<td>3.8</td>
<td>2.4</td>
<td>4.8</td>
<td>2.5</td>
</tr>
<tr>
<td>RBE D37</td>
<td>1.4</td>
<td>1.8</td>
<td>2.6</td>
<td>1.9</td>
<td>2.4</td>
<td>1.9</td>
</tr>
<tr>
<td>RBE D10</td>
<td>1.7</td>
<td>1.6</td>
<td>2.1</td>
<td>1.9</td>
<td>1.9</td>
<td>1.9</td>
</tr>
</tbody>
</table>

                  Cells cultured in normoxia were irradiated in exponential growth phase with carbon-ions (C) and compared to those obtained with x-rays (X). The SF2 is the Surviving Fraction with an irradiation of 2 Gray, $\alpha$ is the linear factor, and $\beta$ the quadratic factor. The ratio $\alpha/\beta$ is the irradiation dose corresponding to an equal contribution of the linear factor $\alpha$ and the quadratic factor $\beta$. The PE is the surviving fraction after 10 days of culture without irradiation, and D37 and D10 are the dose rates corresponding to a surviving fraction of 37% and 10%, respectively. The RBE is the dose rate that leads to the same effect with x-rays and C-ions.
proliferation capability of UM cell lines. On the other hand, when an equal number of cells was plated in the culture dishes, SP6.5 and MKT-BR cells formed equal number of clones between normoxic and hypoxic conditions (PE SP6.5 Hypoxia = 32% versus PE SP6.5 Normoxia = 28%; PEMKT-BR Hypoxia = 24% versus PEMKT-BR Normoxia = 21%). The colony sizes were smaller and their densities lower under hypoxic conditions confirming their reduced growth ability (Fig. 2C, upper part). For all conditions, the percentage of Hoechst-positive apoptotic cells remained under 10% (Fig. 2C, down part). We next concentrated our attention on the radiosensitizer effect of oxygen. We, therefore, compared the SP6.5 and MKT-BR survival curves after x-ray exposure in the normoxic versus hypoxic conditions (P > 0.5, Fig. 2D). These data showed that under hypoxia, UM cell lines become unprotected from x-rays irrespective of the dose selected. A similar conclusion was drawn from other UM cell lines (92.1 and MEL270; Supplementary Fig. S1). It is noteworthy that in other cell types, such as glioblastoma cell lines, similar hypoxic conditions induced a radioresistance (Myriam Bernaudin, personal communication). Interestingly, these data clearly indicated that reduction of the oxygen level in the culture medium is not involved in UM cell lines radioresistance suggesting that another molecular mechanism is required.

Comparison of Cellular Radiosensitivity Toward C-Ions and X-rays

Clonogenic assays were performed to determine the radiosensitivity of UM cell lines (SP6.5, MEL270, MKT-BR, and 92.1) as well as NCM and ARPE19 cells exposed to either C-ions or x-rays. Figure 3 shows the comparison of survival curves that were established from colony formation after C-ions or x-ray radiation. The surviving fractions for all C-ions-irradiated cells decreased exponentially with increasing doses following the radiobiological model of one-hit target (Fig. 3A). Table 2 and Figure 3B show the values of surviving fractions at the same dose of 2 Gy (SF2) for x-rays and C-ions. Interestingly, the SF2 for NCM was very similar between x-rays and C-ions exposure (SF2 x-rays = 0.259; SF2 C-ions = 0.226, P = 0.67), whereas it was significantly lower after C-ions than x-ray radiation for all UM cell lines. The SF2 values for the UM cell lines ranged from 0.448 (SP6.5) to 0.680 (MKT-BR) after x-ray radiation and decreased to 0.139 to 0.360 after C-ions radiation. Therefore, we concluded that UM cell lines are distinctly more sensitive to C-ions than to x-rays. Moreover, on the basis of these survival curves, the RBE values were calculated for radiation doses D37 and D10. The RBE D37 was estimated to be near 1.9 to 2.8 for the UM cell lines, whereas RBE D37 values for normal cells (NCM and ARPE19) ranged from 1.4 to 1.8 (Table 2). The RBE D10 values ranged from 1.6 to 2.5 for UM cell lines and normal cells.

Morphologic Changes After Irradiation

Based on the values presented in Table 2, we next irradiated the SP6.5 and MKT-BR cells with the D10% and D10. Therefore, SP6.5 cells were irradiated with 5 and 8 Gy with x-rays and 2 and 4 Gy with C-ions. The MKT-BR cells were irradiated with 6 and 10 Gy with x-rays and 3 and 6 Gy with C-ions. The radiation-induced influence on the morphologic changes of UM cell lines then was studied 5 days after irradiation. Compared to nonirradiated or 2 Gy-irradiated cells, morphologic changes, including enlarged and flattened cell shape with abundant cytoplasm, were markedly apparent in cells irradiated at 4 Gy using both types of irradiation with more aberrant changes after C-ions radiation (Fig. 4), suggesting that this type of irradiation is more effective than x-rays at altering UM cell’s morphology.

Analysis of ERK1/2 After X-rays and C-Ions

Because the ERK1/2 transduction pathway is altered in UM, we next compared the status of ERK1/2 activation after C-ions and x-ray radiation. The MKT-BR and SP6.5 cell lines were irradiated with doses of x-rays and C-ions producing a 1%
survival rate and extracted the proteins over a 6-day period (144 hours). The levels of expression of total ERK1/2 were reduced in SP6.5 after x-ray irradiation. In contrast, the levels of expression of total ERK1/2 were unaffected after C-ions irradiation for SP6.5 and MKT-BR (Figs. 5A, 5B). Interestingly, C-ions irradiation reduced ERK1/2 phosphorylation levels by 50% from 6 hours until 144 hours in both cell lines (Figs. 5A, 5B). Moreover, the amplitude of p90RSK phosphorylation correlated with the degree of ERK1/2 activation after C-ions: its kinetics matched those of decreased-ERK1/2 activation in SP6.5 and MKT-BR cells. These results indicated that C-ions were involved in deactivation of ERK1/2-p90RSK signaling.

Figure 2. Factors inducing x-ray resistance in UM cell lines. (A) Comparison of x-ray sensitivity between asynchronous cells (solid lines) and G0/G1 arrested cells (dotted lines). Values represent the mean ± SEM of three independent experiments. (B) Comparison of UM cell lines proliferation in normoxia and hypoxia. The SP6.5 and MKT-BR lines were cultured for 6 days under normoxic (21% O2, solid lines) or hypoxic (1% O2, dotted lines) conditions. Data show the proliferation ratios (counted cells/seeded cells) and represent the mean ± SD of three independent irradiation experiments. (C) Effects of hypoxia in clonogenic assays. Representative photographs of colonies yielded by SP6.5 and MKT-BR cells cultured for 10 days under normoxic and hypoxic conditions (upper panel). Illustrative photomicrographs of Hoechst staining of SP6.5 and MKT-BR cells 48 hours after incubation in normoxic and hypoxic conditions (lower panel). Magnification: ×100. (D) Influence of hypoxia on x-ray sensitivity. Clonogenic survival curves following x-ray exposure of SP6.5 and MKT-BR cells under normoxic (21% O2, solid lines) and hypoxic (1% O2, dotted lines) conditions. Cells were irradiated in exponential growth phase. The survival curves were fitted according to the linear quadratic equation (S = e(−αD − β D2)). Results are expressed as mean ± SEM from three independent irradiation experiments.
pathway compared to x-rays and, therefore, suggested that a C-ions-specific mechanism altered this transduction.

**DISCUSSION**

In this study, we evaluated the radiosensitivity of UM cell lines and compared the ERK1/2 expression after x-ray or carbon-ions (C-ions) radiation. Limited data have been published on the x-ray radiosensitivity of UM cell lines. Moreover, studies on C-ions radiation are limited primarily because of the difficulty of accessing a C-ions accelerator facility. Interestingly, C-ions share the valuable physical properties of protons but also have a biological advantage in that their effectiveness increases at the end of the beam's range, whereas it is low along the entrance channel. Moreover, the biological advantages of carbon ions over protons are expected to be most pronounced for tumors that demonstrate low radiosensitivity when treated with photons. As a matter of fact, the RBE of carbon ions is greater than that of protons. Thus, the clinical interest lies in the existence of differential effects between the tumor and normal tissue that favors the latter.

![Comparison of cell survival curves following exposure to x-rays and C-ions.](https://iovs.arvojournals.org/pdfaccess.ashx?url=data/journals/iovs/933929/)
FIGURE 4. Influence of x-rays and C-ions on the morphological characteristics of UM cell lines. Representative phase-contrast photomicrographs of the morphological changes in SP6.5 and MKT-Br cells 5 days after irradiation (0, 2, and 4 Gy) with x-rays (X) and C-ions (C). Scale bar: 50 μm.

A. Time (Hrs): 0 6 24 48 144

X-rays

Dose for SF 1%

Sp.65

C-ions

ERK1-P

ERK2-P

ERK1

ERK2

P pS6

Actin

MKT-Br

B. Sp.65

Relative amount of ERK1/2 activation

0 6 24 48 144

Time (Hrs)

MKT-Br

Relative amount of ERK1/2 activation

0 6 24 48 144

Time (Hrs)
Fig. 5. X-rays and C-ions differentially affect ERK1/2 activation in UM cell lines. (A) The SP6.5 and MKTBR cells exposed to radiation doses that support 1% of survival fraction (i.e., Table 2) were lysed at the indicated time (0 to 144 hours) after irradiation. Proteins were reduced and subjected to SDS-PAGE and Western blot analysis with specific anti-phospho-ERK1/2, anti-total ERK1/2 and anti-phospho-p90RSK. Actin expression also was monitored as a normalization control. Similar results were obtained in three independent experiments. (B) Quantification of the phospho-ERK1/2 signals from (A). Data are means ± SD (n = 3).

group published data on C-ions irradiated UM cell lines.38 Their study revealed that exposing the UM cell lines 92-1 and OCM-1 to x-ray and C-ions radiation resulted in significant differences in term of radiosensitivity, but similarities in term of their respective DNA repair ability.38 Our study is the first to our knowledge that compares survival, effects under hypoxia and impact on ERK levels of phosphorylation in UM cell lines subjected to C-ions and x-ray radiation. Although our data cannot be extended directly to clinical practice, the comparison of x-rays to C-ions is relevant for future development of radiotherapy in UM.

A hallmark component of classical radiobiology, the α/β ratio, is one of the overriding parameters used to model cell killing by radiation. It is the byproduct of the linear quadratic model, which describes cell killing as a single hit versus the double hit hypothesis, where linear cell kill is expressed by the α component, while quadratic cell kill is expressed by the β component.31 The ratio is obtained from isoeffect curves plotted using the survival fractions (SF2) of a single cell line at different doses per fraction.59 For low α/β tumors, C-ions radiation could eliminate the relative radioresistance to photons treatment by decreasing the predominance of the β-component, and subsequently decreasing also the capability for sublethal damage repair.11 Cell survival curves parameters indicated that α-component values for all UM cell lines were lower than those of no tumoral cells (NCM and ARPE) suggesting that UM cell lines are relatively x-ray resistant, especially at lower doses, compared to normal cells. Interestingly, MKTBR, the most x-ray-resistant cell line, showed similar values of cell survival compared to the most x-ray sensitive cell line SP6.5, indicating no difference in the C-ions sensitivity between these cell lines that also bear the B-RafV600E mutation. Moreover, the RBE was similar in the MEL270 cell line that also has the GNAQ209P mutation. Several factors, such as cell cycle distribution, DNA repair system, and level of oxygenation may explain this x-ray resistance.40–42 Our experimental findings from synchronous and asynchronous normal and tumoral melanocytes showed surprisingly no difference in the x-ray sensitivity indicating that cell cycle distribution is not involved in the resistance of UM cell lines toward x-rays.

We demonstrated that UM cell lines survival curves after x-ray radiation were similar in normoxic and hypoxic conditions, indicating that the radioresistance observed does not depend on the level of oxygen. Identifying hypoxia-induced genes and downstream signaling molecules associated with radioresistance may help in determining which types of tumors would benefit from C-ions radiation. A plethora of evidence has shown the increasing importance of the heterodimeric transcription factor hypoxia-inducible factor 1 (HIF-1) in hypoxia.43 However, our in vitro results can be linked to previous studies showing no key function for HIF-1α in irradiated UM tumors.44,45

By controlling cyclin-dependent kinases, one can develop a mechanism to explain the low radiosensitivity of different UM cell lines and provide radiobiological evidence as to why either higher doses of radiation may overcome potentially lethal damages.46 Thus, we hypothesize that x-ray resistance may be linked to the sustained activation of the ERK1/2 pathway in UM. We have shown previously that UM cell lines display constitutively high levels of ERK1/2 activation, which may be explained for some of them by the expression of either B-Raf, GNAQ, or GNA11 mutants.20,28,29 We found that the levels of expression of total ERK1/2 were unaffected during both types of irradiation. Several studies have reported that x-ray ionizing radiations can activate ERK1/2.26,47 We also found that irradiation with C-ions reduced ERK1/2 phosphorylation levels by 50%. It has been shown that only very low doses of ionizing radiation stimulate ERK1/2, and enhance cell proliferation, whereas higher doses activate p53 that, in turn, antagonizes the proliferative effect of ERK1/2 activation and results in cell cycle arrest.47 Another explanation could be the carbonylation of proteins after C-ions irradiation, which affects the phosphorylation of proteins involved in ERK1/2-p90RSK signal transduction. Recently, Asnaghi et al.48 observed that culturing UM cells in hypoxia for 24 hours also activated ERK1/2 proteins. It would be of interest to compare ERK1/2 level of activation after irradiation under normoxia and hypoxia.

Our results also demonstrated that C-ions radiation induced a higher level of clonogenic cell death compared to x-rays and presented an RBE of 1.45 for the NCM and 2.58 to 2.78 for all UM cell lines. Interestingly, we also observed that the survival fraction at 2 Gy (SF2) was similar for NCM between x-ray and C-ions radiation, whereas it decreased after C-ions for UM cell lines. Taken together, these data demonstrated that irradiation with C-ions is more efficient in the treatment of UM cells than x-rays. Such efficiency likely resulted from a more efficient mechanism of cell death with C-ions than with x-rays in UM cells. Further studies will be needed to provide higher quality comparative evidence.

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References

Radiation Sensitivity of Uveal Melanoma


