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BIOLOGY CONTRIBUTION

OUT-OF-FIELD CELL SURVIVAL FOLLOWING EXPOSURE TO INTENSITY-MODULATED RADIATION FIELDS

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Purpose: To determine the in-field and out-of-field cell survival of cells irradiated with either primary field or scattered radiation in the presence and absence of intercellular communication.
Methods and Materials: Cell survival was determined by clonogenic assay in human prostate cancer (DU145) and primary fibroblast (AGO1552) cells following exposure to different field configurations delivered using a 6-MV photon beam produced with a Varian linear accelerator.
Results: Nonuniform dose distributions were delivered using a multileaf collimator (MLC) in which half of the cell population was shielded. Clonogenic survival in the shielded region was significantly lower than that predicted from the linear quadratic model. In both cell lines, the out-of-field responses appeared to saturate at 40%–50% survival at a scattered dose of 0.70 Gy in DU-145 cells and 0.24 Gy in AGO1522 cells. There was an approximately eightfold difference in the initial slopes of the out-of-field response compared with the α-component of the uniform field response. In contrast, cells in the exposed part of the field showed increased survival. These observations were abrogated by direct physical inhibition of cellular communication and by the addition of the inducible nitric oxide synthase inhibitor aminoguanidine known to inhibit intercellular bystander effects. Additional studies showed the proportion of cells irradiated and dose delivered to the shielded and exposed regions of the field to impact on response.
Conclusions: These data demonstrate out-of-field effects as important determinants of cell survival following exposure to modulated irradiation fields with cellular communication between differentially irradiated cell populations playing an important role. Validation of these observations in additional cell models may facilitate the refinement of existing radiobiological models and the observations considered important determinants of cell survival. © 2011 Elsevier Inc.

Out-of-field, Modulated fields, Cell survival, Bystander.

INTRODUCTION

The delivery of clinical radiotherapy is assumed to result in a radiobiological response within the target tumor volume that is proportional to the dose delivered (1). In advanced megavoltage radiotherapy techniques such as intensity-modulated radiation therapy (IMRT), sequential delivery of highly modulated beam profiles are used, resulting in a high degree of dose conformity to the target volume and reducing the dose and risk of complication to normal tissue. Even in the most conformal of treatments, regions of significant dose can accumulate out-of-field because of scattered photons, which may have an impact on cellular response in these regions. With more advanced radiotherapy delivery techniques in clinical use, a more comprehensive understanding of beam quality and its effect on biological responses in and out of the primary treatment field is necessary.

Differences in beam quality outside the primary treatment field for 6-MV photons have been reported in several Monte Carlo studies (2, 3). Kirby *et al.* (2) demonstrated a significant increase in the low energy component of the fluence spectra outside of the primary field corresponding to increased linear energy transfer (LET). Similarly, Liu and Verhaegen (3) showed a 20% variation in beam quality comparing penumbra and central axis. In contrast, Moiseenko *et al.* (4) showed no significant difference in beam quality between central axis

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and penumbra regions of a tomotherapy fan beam out to a distance of 0.6 cm. Recent *in vitro* experimental evidence has shown significant enhancement of DNA damage out-of-field in normal human fibroblasts irradiated with a 6-MV beam (5).

In addition to differences in quality of the beam, communication between irradiated and nonirradiated cell populations through the radiation induced bystander effect (RIBE) may affect biological response (6). Because IMRT beams are by definition spatially modulated, cell communication between differentially irradiated cells populations within the target tumor volume may also have an important role.

Several *in vitro* studies have attempted to address this question (7–10). Using a wedge to create a nonuniform field, Suchowerska *et al.* (7) observed differences in survival response between cell populations in which intercellular communication was either intact or physically inhibited (7). Differences in cell survival were also shown by Claridge Makonis *et al.* (8) by comparing delivery of a uniform field with delivery to 25% of the cell population as a single region or as three parallel stripes within the same flask. Moiseenko *et al.* (9) reported reduced cell kill for IMRT treatment plans compared with acute irradiation for head and neck treatment plans delivered *in vitro*.

Recent evidence from our laboratory showed no significant difference in the survival response following exposure to modulated and nonmodulated 6-MV fields under conditions in which modulation was delivered as a series of step functions across the cell population (10). However, it is difficult to draw conclusions from these reports because of differences in the spatial and temporal components of modulated beam delivery, with protracted delivery times associated with reduced cell kill (11–13).

In this study, we determined the survival responses in field and out of field for a modulated 6-MV photon beam in a human normal and tumor cell line using a multileaf collimator (MLC) to define the in-field exposed area. The effect or intercellular communication between the in and out-of-field cell populations was investigated. Additional studies were performed varying the proportion of cells in and out of the primary field.

METHODS AND MATERIALS

Cell culture

Experiments were conducted using two cell lines, the human prostate cancer cell line, DU-145, and the human fibroblast cell line, AGO-1552. Cell lines were obtained from Cancer Research UK and selected as malignant and transformed models with different radiosensitivity. DU-145 cells were grown in RPMI-1640 with L-glutamine (Lonza, Cambridge, United Kingdom) supplemented with 10% fetal bovine serum, 1% penicillin/streptomycin (Gibco, Paisley, United Kingdom). AGO-1522 cells were grown in Eagle's minimum essential medium with deoxyribonucleosides and deoxyribonucleotides (Lonza) supplemented with 20% fetal bovine serum and 1% penicillin/streptomycin. All cell lines were maintained at 37°C in a humidified atmosphere of 5% CO_2.

Clonogenic assay

Cell survival was determined by clonogenic assay as previously reported (10). Cells were plated and allowed to adhere overnight. Culture flasks were filled with serum-free medium and sealed immediately before irradiation. Cells were irradiated at room temperature (25 ± 2 °C). Following irradiation, serum-free medium was removed and replaced with complete culture medium. Cultures were incubated for 10–14 days before staining with 0.5% crystal violet in 50% methanol. DU-145 colonies were scored using a Colcount (Oxford Optronix, United Kingdom) automated counter which optimized for the cell line. AGO-1522 colonies were scored manually applying a 50-cell exclusion rule. For each experiment, unexposed controls were prepared and treated as sham exposures. Experiments were conducted under standard culture conditions or in the presence of the inducible nitric oxide synthase (iNOS) inhibitor aminoguanidine (AG) at a concentration of 100 μM. AG was diluted in phosphate-buffered saline to the desired final concentration and added to culture medium 2 hours before irradiation. AG remained present in the culture medium for the duration of the assay.

Irradiation setup and validation of experimental design

Cells were irradiated in either T75 or T25 culture flasks (Nunc, Loughborough, United Kingdom) with a 6-MV photon beam produced by a Varian 600CD medical linear accelerator with 120-leaf millennium MLC (Varian Medical Systems, Palo Alto, CA) calibrated according to the UK Code of Practice (Institute of Physical Sciences in Medicine, 1990).

The same experimental setup was used as previously described (10). Full scatter conditions were achieved by filling the flask with culture medium before irradiation and submersing in a water phantom on top of a 30 × 30 cm, 5-cm-deep block of solid water that was placed on the treatment couch. The gantry was placed at 180°, and the couch was placed so that the source to surface distance to the couch top was 100 cm. All calculations for set monitor units included a factor to account for the attenuation of the couch. The setup was CT scanned using a Siemens Emotion 6 (Siemens, Erlangen, Germany), and a plan was created using Nucletron Oncentra (Nucletron, Veenendal, the Netherlands) to ensure uniform irradiation of the culture flask using a 20 × 20 cm field gave the number of monitor units required to give the prescribed doses to cells in the flasks of (0–6.28 Gy) for AGO and (0–12.40 Gy) for DU145 cells. Plans were also created with MLCs shielding 50% of the 20 × 20 cm field, and monitor units were calculated to give equivalent doses to the exposed area as was given for the open field.

MLC leaf transmission and leakage is known to be higher than for standard collimators. To confirm that this was not affecting the results, the secondary collimators were used to shield half of the 20 × 20 cm field in place of the MLC leaves. Using the secondary collimators to deliver a nonuniform field resulted in a lower scattered dose of 0.39 ± 0.08 Gy being delivered compared with 0.47 ± 0.09 Gy when using the MLC (due to transmission through the MLC). However, both the in-field and out-of-field survival responses when using the secondary collimators showed no significant difference in response than when using the MLC as shielding. Figure 1 shows that the out-of-field region corresponded to the neck of the T75 flask. To confirm that this was not adversely affecting the data, the collimator was rotated by 180°, and the 8 Gy was delivered in-field to the neck end. The delivery of the same dose to the flask in the opposite orientation had no significant impact on the relative survival responses in field and out of field.

A number of measurements were performed to ensure that the T75 flask was receiving the correct absolute dose using Gafchromic