Abstract. Charged particle therapy utilizing protons or carbon ions has been rapidly intensifying over recent years. The present study was designed to jointly investigate these two charged particle treatment modalities with respect to modeled anatomical depth-dependent dose and linear energy transfer (LET) deliveries to cells with either normal or compromised DNA repair phenotypes. We compared cellular lethality in response to dose, LET and Bragg peak location for accelerated protons and carbon ions at 70 and 290 MeV/n, respectively. A novel experimental live cell irradiation OptiCell™ in vitro culture system using three different Chinese hamster ovary (CHO) cells as a mammalian model was conducted. A wild-type DNA repair-competent CHO cell line (CHO 10B2) was compared to two other CHO cell lines (51D1 and xrs5), each genetically deficient with respect to one of the two major DNA repair pathways (homologous recombination and non-homologous end joining pathways, respectively) following genotoxic insults. We found that wild-type and homologous recombination-deficient (RAD51D) cellular lethality was dependent on both the dose and LET of the carbon ions, whereas it was only dependent on dose for protons. The non-homologous end joining deficient cell line (Ku80 mutant) showed nearly identical dose-response profiles for both carbon ions and protons. Our results show that the increasingly used modality of carbon ions as charged particle therapy is advantageous to protons in a radiotherapeutic context, primarily for tumor cells proficient in non-homologous end joining DNA repair where cellular lethality is dependent not only on the dose as in the case of more common photon therapeutic modalities, but more importantly on the carbon ion LETs. Genetic characterization of patient tumors would be key to individualize and optimize the selection of radiation modality, clinical outcome and treatment cost.

Introduction

Charged particle therapy research and its clinical application has been expanding since its introduction in the early 1960’s. Today, proton therapy is the prevailing form of charged particle therapy with 37 facilities around the world treating patients with various types of cancers including uveal melanoma, unresectable sarcomas, and basal skull or paraspinal tumors that require a significantly higher dose of ionizing radiation (1-4). Proton therapy is also considered advantageous relative to conventional forms of photon radiotherapy in cases where precise localization of the radiologic effects to a tumor is imperative during treatment. Quintessential examples of the types of tumors where proton therapy is the most advantageous in treatment include prostate cancer and pediatric neoplasms (5-7).

In the 1990’s, the advantages of proton therapy eventually led to an expansion of the field of charged particle therapy to include carbon ion radiotherapy. Today, carbon ion radiotherapy centers at a limited number of locations worldwide are currently treating patients for the same types of cancers commonly treated elsewhere with protons (8-14). Carbon ions are typically accelerated at energies between 140 and 400 MeV for applications in a clinical setting whereas energies between 65 and 200 MeV are most commonly utilized when accelerating protons (11,15). Proton and carbon radiotherapy are both effective for precisely treating and delineating a localized tumor during treatment with ionizing radiation. This is due to the beam of accelerated particles gradually depositing increasing amounts of energy along a path in the biological
suggest that carbon ion therapy is advantageous over protons in intermediate sensitivity to protons. Collectively, our findings indicate that the cellular lethality that was completely dependent on dose regardless of the type of radiation exposure. Conversely, wild-type cells, which were sensitive to carbon ion exposure, least sensitive to γ-ray exposure, and showed intermediate sensitivity to protons. Collectively, our findings suggest that carbon ion therapy is advantageous over proton therapy in light of carbon ion irradiation characteristics. Most noteworthy are the higher LET values at the Bragg peak and the fact that LET levels themselves in combination with dose are primary determinants of cellular lethality when treating a tumor. Ultimately, the specific genetics of any given malignancy with respect to its DNA damage repair proficiency affects the final extent of therapeutic advantage gained through the use of carbon ion beams in patients treated at charged particle therapy centers.

Materials and methods

Radiation conditions. Particle-based irradiation experiments were carried out at the NIRS in Chiba, Japan. Carbon ions were accelerated to 290 MeV/n using the Heavy Ion Medical Accelerator (HIMAC) synchrotron and protons were accelerated to 70 MeV/n using the NIRS-930 cyclotron delivery port in C-8. Dose rates for carbon ions and protons were set at 1 Gy/min. γ-ray irradiation experiments were carried out at a dose rate of ~2.5 Gy/min at Colorado State University (Fort Collins, CO) using a Model Mark I-68A (SS0056) 6,000Ci 137Cesium sealed source model (J.L. Shepherd, Carlsbad, CA). X-ray irradiation experiments were carried out at the NIRS using a Titan X-ray generator (Shimadzu, Japan) with a peak tube/voltage potential of 200 kVp, a tube intensity of 20 mA, 0.5 mm of aluminum and copper filters, at a dose rate of 1 Gy/min. Irradiations were carried out at room temperature.

Cell culture. Original Chinese hamster ovary epithelial wild-type cells (CHO 10B2) were kindly supplied by Dr Joel Bedford (Colorado State University, Fort Collins, CO). DNA repair-deficient CHO mutant cell lines for the: i) homologous recombination pathway (51D1 cells; CHO AA8 RAD51D mutant cell lines) and ii) the non-homologous end-joining pathway (xs5 cells; Ku80 gene deficient) were kindly supplied by Dr Larry Thompson (Lawrence Livermore National Laboratory, Livermore, CA)(26,27). All cells were grown and maintained in α-MEM (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum (FBS, Sigma, St. Louis, MO), 1X antibiotics and antmycotics (anti-anti, Invitrogen), at 37˚C in CO₂ incubators at 5% CO₂ and 100% humidity. Doubling times were ~12 h for all cell lines.

Irradiation procedure and cell survival assays. Cultured cells were trypsinized and re-suspended into growth medium containing α-MEM with 10% FBS and antibiotics (anti-anti, Invitrogen). Once re-suspended, 10 ml of medium containing between 500 and 700 cells were placed into each individual Opticell™ (Thermo Scientific, Rochester, NY) cell culture container ~1 h prior to irradiation. All samples were then appropriately organized and irradiated. Radiation physics quantitative values including dose distribution and LET distribution for both of the proton and carbon beams used is summarized in Fig. 1. Immediately following radiation, all cells were incubated at 37˚C with 5% CO₂ humidity for 7-10 days. After this culturing period, tissue culture vessels were then washed with 0.9% NaCl, fixed in 100% ethanol, and stained with 0.1% crystal violet. Colonies containing >50 cells were scored as a surviving colony. A minimum
of three independent experiments was carried out for each type of radiation studied. Survival curves were drawn for individual data points plotting a given Opticell container’s ‘depth’ from doses calculated according to values presented in Fig. 1.

Data treatment and statistical analysis. All experimental data were analyzed using the Prism 5™ software. Standard errors of the means for all data points were calculated for all experimental data points and are depicted in each figure.

Results

Radiation physics parameters. Depth distribution values for the dose and LET were calculated and plotted against corresponding depths in water for the 70 MeV/n accelerated protons and 290 MeV/n accelerated carbon ions (Fig. 1). These maximum doses were delivered at depths of ~4 and 14 cm (in water), for protons and carbon ions, respectively. In regard to LET distribution values, carbon ions displayed higher LET values at all depths (in water) relative to the protons, and delivered a peak LET at a depth of ~14 cm (in water). Proton LET values were significantly lower relative to those for carbon ions and a peak LET at a depth of ~4 cm (in water).

Dose-depth distribution effect of proton and carbon beam cellular lethality. Stacked Opticell culture system cell survival assays were carried out using CHO wild-type xrs5 and 51D1 cells exposed to 70 MeV/n accelerated protons and doses of 290 MeV/n accelerated carbon ions. Carbon ions yielded lower survival fractions relative to protons at their respective Bragg peaks for wild-type and 51D1 cells at equal and lower doses (Fig. 2). xrs5 cells had comparable survival values for proton and carbon ions at doses of 0.5 and 1 Gy (Fig. 2).

Determination of dose-dependent cell survival. Cell survival was evaluated in response to the dose for all three cell lines using γ-rays, 70 MeV/n accelerated protons, and 290 MeV/n accelerated carbon ions. Both wild-type and 51D1 cells were most sensitive to carbon ions, and displayed responses in cell survival that were dependent on both the dose and LET for this particular form of radiation. In contrast, xrs5 cells displayed relatively comparable cell survival responses to dose for all types of radiation exposure, including exposure to high LET (defined as LET values >30 keV/µm) carbon ions (Fig. 3).

RBE values for wild-type cells. RBE values were calculated based on the average D10 values representative of a particular form of radiation. High LET 290 MeV/n carbon ions had the highest average RBE of 2.03, followed by low LET 290 MeV/n carbon ions with an RBE of 1.29 and 70 MeV/n protons with an RBE of approximately 1.

Discussion

Our results show that the degree of cell killing assessed via cell survival assays was dependent on the dose for all types of radiation used in our study (Fig. 3). Of the various types of radiation used, carbon ions produced cell survival levels that were dependent on both the dose and amount of LET exposure (Fig. 3). The LET values of carbon ions near Bragg peak are about one hundred times higher than protons and other low LET radiation values (Fig. 1). In regards to notable differences observed in survival responses between the different cell lines used, wild-type and homologous recombination mutant 51D1 cells tended to be the most sensitive to carbon ion radiation, especially at regions of high LET (Figs. 2 and 3). On the other hand, xrs5 cells displayed essentially the same sensitivity to the three types and LET radiation used in our study (Figs. 2B and 3). At lower doses (of 1 Gy) in particular, non-homologous end joining deficient cells trended towards a higher sensitivity to either carbon ions or protons than did homologous recombination mutants, which is consistent with...
Figure 2. (A) DNA repair-competent cell survival vs. depth in water. Wild-type mammalian epithelial CHO 10B2 cells were exposed to 70 MeV/n accelerated protons and 290 MeV/n accelerated carbon ions. Carbon ion exposure resulted in notably lower survival fractions at Bragg peak regions relative to protons at entering doses of 1 and 2 Gy. (B) DNA repair-deficient cell survival vs. depth in water 51D1 and xrs5 repair-deficient cell lines were exposed to 70 MeV/n accelerated protons and 290 MeV/n accelerated carbon ions. Both beams yielded similar survival fractions at doses of 0.5 and 1 Gy at their respective Bragg peaks for xrs5 cells. 51D1 cells were considerably more sensitive to carbon ions at Bragg peak regions when compared to protons at entering doses of 1 and 3 Gy.
When comparing the effectiveness between carbon ion radiotherapy and proton radiotherapy, our study suggests that carbon ions are advantageous to protons in the sense that cellular lethality is dependent on both dose and LET values for carbon ions (for cells proficient in non-homologous end joining DNA repair), whereas cell survival for protons is dependent only on dose. Additionally, carbon ions in our study displayed LET values that were significantly higher than protons at the Bragg peak regions, which is also advantageous, especially when considering that an SOBP of high LET radiation is actually administered to a tumor as a whole.

A previous study reported findings similar to ours when evaluating the cellular lethality for xrs5 cells in response to increasing LET exposure of carbon ions (28). Concurrently, these two studies suggest that loss of non-homologous end joining DNA repair capacity undermines the carbon ion cellular lethality dependence on both the quality of radiation and quantity of LET exposure, and that only patients diagnosed with NHEJ DNA repair-competent tumors will maximally benefit from carbon ion therapy. To the best of our knowledge,
our present study is one of the first to investigate whether cells deficient in the other major cellular DNA repair pathway and incapable of homologous recombination also display this phenomenon. Results from the present study demonstrate that homologous recombination-deficient cells demonstrate a dependence on the quality of radiation and quantity of LET when cell survival is measured. However, investigative efforts are still required to definitively reiterate or reinforce this finding.

Another finding from our study that is worth mentioning are the RBE values derived from D10 values representing our monoenergetic proton and carbon beams. We discovered average RBE values calculated from D10 doses to be 2.03 at high LET Bragg peak regions and 1.29 at low LET regions outside the Bragg peak for wild-type cells exposed to carbon ions. The average calculated RBE for protons was approximately 1.0 for wild-type cells. From the perspective of RBE in our study, carbon ions may be considered advantageous to protons when using this wild-type in vitro model. RBE values for wild-type cells in our study are comparable to those depicted in previous studies (15,29,30).

In light of our findings, it would be significant to determine if carbon ions remain advantageous over protons in terms of a LET deposition and cell survival dependence on both dose and LET deposition at lower energy levels where a Bragg peak for accelerated carbon ions is located at the same depth in water as a comparative proton beam. Additionally, a future investigative effort evaluating the degree of synergy between inhibitors of various DNA repair pathways (i.e. homologous recombination and non-homologous end joining repair) combined with either proton or carbon radiation exposure could shed light on our findings. Further research involving proton and carbon in vitro experiments and more effective particle therapy treatment modalities are required for types of localized cancers where these types of radiation are applicable.

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References