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EDITORIAL

Preclinical Models for Translational Research Should Maintain Pace With Modern Clinical Practice

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The technical evolution of radiation therapy for brain cancer has improved both the efficacy of individual radiation treatments and patient safety. The advent of computed tomography (CT)-based planning marked an important initial shift toward target-directed treatment (1). Treatment planning accuracy was further increased by fusing CT planning images with positron emission tomography (PET) and magnetic resonance imaging (MRI) scans (2, 3). Additionally, cone beam CT images acquired before each fraction were added to compensate for any deviations from the simulation CT (4). These innovations, both in target delineation and in image guidance, have translated into better tumor control and fewer treatment related toxicities (5). Future advances are likely to come from emerging pharmaceutical approaches (6) or direct targeting of biological mechanisms that drive tumor radiation resistance (7). To best facilitate the rapid translation of this new radiobiology, novel approaches need to be validated in preclinical animal models using established clinical procedures.

Cell culture experiments provide basic radiobiological information, such as defining the time course of radiation damage processing and repair. However, the translational relevance of in vitro data to the clinical practice of radiation oncology is limited. Preclinical animal models offer greater potential, particularly when the conditions of clinical practice are mimicked by the use of an orthotopic tumor model or target-directed radiation therapy using a relevant treatment regimen. Innovative radiation therapy devices specifically developed for preclinical models are making this type of research approach now possible (8-10), along with noninvasive methods to define in situ tumor response such as bioluminescence (10-12), use of reporter transgenes (13), optical imaging (14), and microMR/PET/CT imaging (15-18). For example, Baumann and colleagues (10) developed a genetically modified tumor cell line for a bioimageable intracranial tumor, and they used this model along with histologic and other imaging techniques to verify precise preclinical radiation delivery.

There are both advantages and disadvantages to each of the noninvasive imaging methodologies. In all cases, noninvasive imaging allows for each animal to serve as its own control, which permits the tracking of disease progression, akin to the evaluation process used in clinical radiation therapy. However, such an approach does not allow tissue to be removed at each time point of interest, which prevents a histologic correlate being made. Optical, photoacoustic, and ultrasound modalities are all relatively low cost and provide high throughput, but these methods are often limited by poor depth resolution or require genetic manipulation for signal production (19). Therefore, these techniques are limited to mechanistic or pharmacologic studies (20), and the clinical translational opportunity is limited because of the lack of 3D quantification. Small animal imaging using PET, single-photon emission computed tomography (SPECT), CT, or MR is more cost-prohibitive, and scan duration limits high throughput. However, these imaging modalities provide high 3D resolution and sensitivity, and the clinical applicability is evident. For this reason alone, the use of these clinically established techniques for translational studies is more rational (21). However, the merit of combining multiple techniques for the assessment and evaluation of new cancer therapies is likely to be the most promising (22). The advances in preclinical noninvasive imaging as applied in radiobiology have made the use of orthotopic tumor models almost routine.

Many research groups have demonstrated that intracranial brain tumor models can be used to investigate critical aspects of tumor radiobiology (10-17, 23, 24). For example, studies from Stanford have focused on tumor neovascularization after irradiation (11), whereas other groups have focused on stem cell radioresistance (24), tumor invasion, and deregulated angiogenesis

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(11, 12, 15). In our studies, an orthotopic glioblastoma multiforme (GBM) model was utilized to investigate the effects of novel irradiation schemes on tumor microenvironment and surrounding normal brain tissue (15-17). After stereotactic implantation of human GBM tumor cells in nude mice, tumor growth and response to treatment were assessed with weekly CT, [^{18}F]-FDG-PET, and MRI scans. In these initial studies, radiation was administered with a standard 1-beam single-field 160 kVp animal X-irradiator (Faxitron Bioptics, Tucson, AZ) (0.5 mm Cu and Al filters: HVL: 0.77 mm CU). This device, however, has several limitations that create divergence from clinical treatment conditions. First, intracranial irradiation requires meticulous placement of lead canopies to limit dose to surrounding organs (Fig. 1). To ensure adequate coverage of the murine brain, however, this customized shielding still exposes portions of the globes, gastrointestinal and respiratory mucosae, and salivary glands. Prescription doses, when escalated toward therapeutic levels used in the clinic, may generate sufficient exit dose to exceed tolerance of these tissues, causing toxicities that hinder physiologic functions, such as feeding. A second limitation is the lack of image guidance because treatment setup is based on external anatomic landmarks. Although the location of intracranial contents in toto is relatively predictable and is well approximated by the globes and occiput, targeting specific portions of the brain is problematic. Appreciating the precise location and geometry of tumors elsewhere in the body is more difficult, particularly when the tumors are not palpable. Third, a photon beam attenuates as it traverses matter and inevitably leads to dose heterogeneity. This phenomenon is more pronounced with lower energy beams and is unavoidable with single-beam treatment.

These limitations can be overcome by the use of dedicated 3-dimensional (3D) conformal small animal microirradiator devices such as the Small Animal Radiation Research Platform (SARRP, Xstrahl, Gulmay Medical Inc, Suwanee, GA) (9, 10) or small animal irradiation system (XRad225Cx, Precision X-Ray, Inc, North Branford, CT) (8, 14). Such machines provide a sophisticated method of delivering radiation in the research setting and reconcile many of the concerns associated with standard preclinical radiation devices. For example, a rotatable gantry and interchangeable collimators of various sizes and dimensions allow irradiation of anatomic subsites without shielding, and the close proximity of the collimator to the target minimizes the beam's physical penumbra (Fig. 1). For the SARRP, both the gantry and the platform stage demonstrate 360° of motion and support multiple-beam (coplanar and noncoplanar) and continuous arc treatment with isocenter-based treatment planning (Fig. 2). Crosshairs are placed at the desired location (eg, in the center of a tumor) on a CT image taken at the time of simulation to target each treatment beam. Coregistration of planning CT images with those of PET and MRI scans is also possible. Figure 3 illustrates the utility of T1-weighted MRI sequences (after the administration of intravenous contrast medium) to define the anatomic borders of a tumor and T2-weighted sequences to define pathologic enhancement associated with edema and subclinical disease. Fusing MRI with planning CT images allows superior tumor localization compared with CT alone and mirrors the process of target delineation used in current clinical practice. Functional nuclear medicine scans may also supply additional information regarding tumor growth and oxygenation. Figure 2 illustrates dose distributions that result when 2 Gy is administered using various single-field beams, prescribed to an isocenter placed at the center of an orthotopic GBM tumor. Each beam per se leads to beam heterogeneity, with regions of the brain proximal to the beam entrance receiving higher than the prescribed dose and distal regions receiving lower than the prescribed dose. When a 2-Gy fraction is divided evenly among the 3 fields by the use of an automated rotatable gantry, greater dose homogeneity is achieved, and underdosing and overdosing of tumor is minimized (Fig. 2). Inadvertent radiation exposure of the gastrointestinal mucosa by the exit dose is also minimized.

The technological advantages of image guided radiation delivery for small animals appear to translate into improved tumor control in an orthotopic GBM model. In our studies, mice with implanted, rapidly growing, well-visualized U87-MG tumors were imaged with serial CT and quantified. Tumor growth was assessed and compared weekly after 10 Gy was given each week in daily 2-Gy fractions, Monday through Friday with a weekend gap. Treatment started on day 7 after implantation, with the use of either a 1-beam single field treatment (Faxitron) or the 3-field treatment with image guidance. In comparison with treatment with a single beam, 3-field image guided treatment resulted in a 26%, 69%, and 81% reduction ($P = .1, < .01, < .01$) in normalized tumor volume after the administration of 10 Gy, 20 Gy, and 30 Gy,

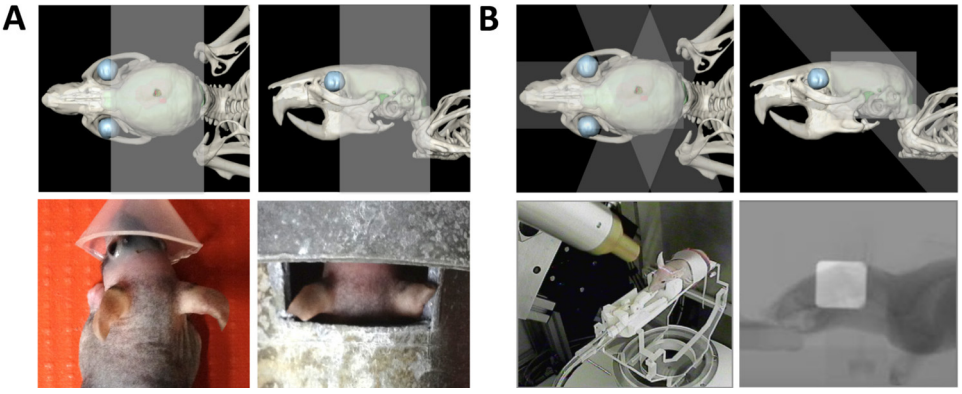

Fig. 1. (A) Schematic representation of the experimental setup required to irradiate using a vertical, single planar beam (upper panels). Experimental view for animal treatment with and without shielding. The lead shielding limits dose to surrounding organs. (B) Schematic representation of the Small Animal Radiation Research Platform (SARRP) beam arrangement on isocentric 3-beam treatment (upper). Image guided cranial irradiation using the SARRP, treatment view (lower left). Mounted photon detectors provide beam's-eye-view portal images, which can be used to verify treatment setup before the initiation of a fraction (lower right).