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Effect of Low-dose Radiation on Cognitive Function of Rats at Different Ages

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Purpose/Objective(s): It is known that radiation can induce cognitive dysfunction, but we still know little about the threshold of radiation dose. It turns out that 0.1 Gy radiation can modulate the expression of gene pathways involved in cognitive function. However, it isn't clear whether 0.1 Gy whole brain irradiation can induce cognitive impairments. In this study, the relationship between 0.1 Gy exposure and cognitive function was investigated in rats at different ages.

Materials/Methods: Thirty-two 1-month-old male Sprague-Dawley rats and thirty two six-month-old male Sprague-Dawley rats were divided randomly into 0.1Gy irradiated group and anesthesia control group. The rats were anesthetized, and then the whole brains were irradiated using 4-MeV electron beams delivered by a linear accelerator. Behavioral tests were performed 3 months after irradiation in the order of open field, Morris water maze and passive avoidance test. The tests were used to evaluate the anxiety level, hippocampal dependent spatial learning and memory, and nonspatial learning and memory respectively. In Morris water maze, the place navigation test was conducted on the 1st to 4th day; while on the 5th day, the spatial probe test was done.

Results: In the place navigation test of Morris water maze, six-month-old rats exhibited age dependent impairments in latency to find the hidden platform compared with the one-month-old rats. But radiation did not affect the six-month-old rats' spatial learning and memory. Irradiated group (one month old) had significantly longer latency time than control group. Radiation did not have the effect on the anxiety level in the open field test, and there was no significant difference between groups in the latency of the passive avoidance.

Conclusions: In present study, the hippocampal dependent spatial learning and memory capacity of the one-month-old rats was impaired by 0.1Gy whole brain irradiation, but it did not have the same effect on the six-month-old rats. These findings strongly suggest that low dose radiation is sensitive to induce cognitive impairments in younger rats.

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Effects of Total Body Irradiation on T-Cell and B-Cell Subsets as Well as Macrophage in Rhesus Macaque

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Purpose/Objective(s): Anti-tumor immunity is known to play a major role in the control of neoplasms *in vivo*, and tumor immunotherapy is rapidly becoming a major part of oncology research and evidence-based practice. However, the effects of radiation therapy on the multiple cell types responsible for cellular and humoral immunity are not entirely understood. The specific aim of the present analysis was to determine the acute effects of mini total body irradiation (TBI) on circulating levels of T-cells, B-cells, and monocytes in rhesus macaques (RMs).

Material/Methods: Four female RMs (8-10 years old) were exposed to 2 Gy ionizing radiation to induce immunosuppression, and this state was subsequently maintained with daily tacrolimus and prednisone treatment. T-cell, B-cell, and monocyte frequencies were determined by flow cytometry. In addition, CD4 and CD8 T-cells as well as B-cells were further subdivided into naive and memory subsets. Relative frequency and proliferative status of these subsets was determined by flow cytometry and by measuring changes in Ki-67 expression levels. The institutional animal care and use committee at ONPRC approved the study.

Results: TBI effectively lymphodepleted the RMs while treatment with tacrolimus and prednisone alone did not. However, B-cells were more effectively eliminated than either CD4 or CD8 T-cells. Interestingly, the levels of circulating macrophage increased dramatically following TBI. Moreover, TBI induced significant changes in plasma levels of both pro- and anti-inflammatory cytokines.

Conclusion: Our preliminary results suggest that TBI has differential effects on the cell types involved with anti-tumor immunity, and may effect humoral immunity and antigen presentation to a greater extent than T-cell mediated immunity.

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Relative Biological Effectiveness of Single-Shot Irradiation With Laser-Driven Nanosecond Proton Bunches

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Purpose/Objective(s): Owing to their superior depth-dose curve, protons are a favorable choice in radiation therapy. High intensity table-top lasers are a proposed mechanism of reducing the cost and space consumed by conventional means of proton acceleration. However, before clinical application of laser-driven protons can be considered, cell experiments with laser-driven beams constitute an important preliminary step to demonstrate the increasing maturity of this technology. Here we present first biological results of single-shot irradiation with laser-driven nanosecond proton bunches.

Materials/Methods: The ATLAS Ti-Sapphire laser delivered 400 mJ in a 30 fs pulse on a diamond-like Carbon target for proton acceleration. Two mini-quadrupole magnets focused the protons over a distance of 1.2 m and acted as an energy filter to produce a quasi-monoenergetic beam with an energy of 5.3 $\pm$ 0.15 MeV. The beam manifested as a line focus of $\sim$0.5 mm in width and 6 mm in length. This was used to irradiate human tumor cells over a dose range of 0.13 to 7 Gy, realized by single laser shots. Due to the inhomogeneous dose distribution across the line focus, full dose response curves for single, nanosecond proton bunches were obtained for each irradiated sample. For obtaining a reference dose-response curve cells were irradiated at the Munich tandem accelerator with the same proton energy using a delivery mode where the radiation was given within 100 ms. For all experiments, HeLa cells were seeded directly onto the cell holder window composed of 6 µm Mylar foil 48 hours prior to irradiation. Cells were fixed 30 minutes after irradiation and stained using Alexa 488

for 53bp1 and Cy3 for γ-H2AX foci. Dose was measured using radiochromic film (Gafchromic EBT2) placed immediately behind the back layer of the cell holder.

Results: Foci (γ-H2AX and 53bp1) were counted after irradiation with laser-driven protons and with protons from the conventional accelerator. An RBE of 1.3 ± 0.3 relative to 200 kV X-rays was determined for the induction of γ-H2AX foci and also for 53bp1 foci for laser-driven protons. These results indicate no substantial difference between laser-driven proton beams and those produced by conventional acceleration means.

Conclusion: The RBE obtained in this study for laser-driven protons is in agreement with RBE values in conventional beams at comparable proton energies. This indicates that no new radiobiological effects are to be expected with nanosecond proton delivery, in line with previous studies in single, nanosecond proton bunches at conventional sources. This confirms that for future applications in radiation therapy, the same RBE as for conventional sources can be assumed.

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The Role of XRCC4 in Sensitizing Human Colon Cancer Stem-like Cells to X-ray or Carbon Ion Beam

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Purpose: XRCC4, a member of NHEJ for double strand breaks, is very important in maintaining the overall genome stability, and may play an important role in carcinogenesis. Inactivation of XRCC4 is associated with various human carcinogenesis. Here we try to explore the relationship between XRCC4 and radiosensitivity of human colon cancer stem-like cell to X-ray or carbon ion beam.

Methods: Human colon cancer stem-like cells sorted from HCT116-wild type (WT) and HCT116-XRCC4 knockout (KO) cells were treated with or without carbon ion or X-ray irradiation and then colony formation assay, spheroid formation assay, FACS analyses were performed.

Results: FACS analysis showed that the percentage of CD133+, CD44+ and ESA+ cells in HCT116-WT cells were 3.2%, 6.8%, and 7.2%, whereas 1.6%, 19.2% and 20% in HCT116-XRCC4 KO cells. The proportion of CD133+ and CD44+ cells was increased 3 to 4-fold after 2 Gy X-ray irradiation in HCT116-WT cells, in comparison, 4 to 6- fold increment of CD133+ and CD44+ cells was induced in HCT116-XRCC4 KO cells. In contrast, there was no significant difference of CD133+ proportion between HCT116-WT and HCT116-XRCC4 KO cells after carbon ion beam irradiation. There was no change in proportion of ESA+ cells in HCT116-WT cells, but 10-fold enhancement of ESA+ cells was induced in HCT116-XRCC4 KO cells by 2 Gy of X-ray, whereas only 2-fold enhancement of ESA+ cells was induced in HCT116-XRCC4 KO by isoeffective 1 Gy of carbon ion beam. Colony and spheroid formation from CD133+, CD44+/ESA+ cells were higher compared to CD133-, CD44-/ESA- cells in HCT116-XRCC4 KO cells, but extremely decreased compared to HCT116-WT cells. Colony formation ability of CD133+, CD44+/ESA+ cells delivered from HCT116-XRCC4 KO cells was dramatically lower than that from HCT116-WT cells after either X-ray or carbon ion beam.

Conclusions: Taken together, the expression of cancer stem-like cell markers significantly increased by X-ray compared to carbon ion beam, and XRCC4 inactivation remarkably radiosensitized human colon cancer stem-like cells, suggesting that XRCC4 may play a crucial role in maintaining cancer stemness.

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Correlation of Brachial Plexus Dose With Gross Tumor Volume in Head and Neck IMRT

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Purpose/Objective(s): The purpose of this retrospective review was to determine if the gross tumor volume (GTV) of the primary and nodal disease contributes to the brachial plexus (BP) dose in head and neck cancer (HNC) patients treated with intensity-modulated radiation therapy (IMRT).

Materials/Methods: From 2004 to 2011, 76 HNC patients underwent curative intent IMRT to a total dose of 69.96 Gy in 33 fractions, with the right and left BP prospectively contoured as separate organs at risk (OARs) for a total of 152 brachial plexuses. During IMRT optimization, the intent was to keep the maximum BP dose ≤60 Gy, but with priority given to tumor coverage. Primary gross tumor (GTV-P) and nodal gross tumor (GTV-N) volumes obtained from the dose volume histograms were compared with BP dose parameters.

Results: The mean BP volume was 8.4 ± 4.5 cc, maximum BP dose was 59.6 ± 11.4 Gy, and the mean BP dose was 43.7 ± 10.0 Gy. The mean GTV-P was 36.6 ± 49.9 cc and the mean GTV-nodal was 16.8 ± 52.5 cc. The maximum dose to the BP was ≤60, ≤66, and ≤70 Gy in 70 (46.1%), 123 (80.9%) and 133 (87.5%) BPs, respectively. A greater BP maximal dose of >66 Gy correlated with a larger mean nodal GTV (46.0 cc versus 9.9 cc, $p = 0.0008$), and when dichotomized by mean nodal GTV (16.8 cc) significant differences were noted in BP maximum dose (63.9 versus 58.6 Gy, $p = 0.020$), mean BP dose (47.2 versus 42.8 Gy, $p = 0.033$), and minimum BP dose (32.2 versus 24.6 Gy, $p = 0.0004$) for ≥16.8 and <16.8, respectively. These differences retained significance on multivariate analysis after adjusting individually for BP volume, GTV-P, the use of a Low Anterior Neck (LAN) field technique, total prescription dose, and T category. For GTV-P, a greater BP maximal dose of >66 Gy correlated with a larger mean GTV-P (67.2 cc versus 29.3 cc, $p = 0.0002$). A subset analysis by cancer site (stratified by proximity to the BP) revealed: a BP maximum dose of >66 Gy was associated with larger GTV-P for tumors of larynx, hypopharynx and oropharynx (70.3 cc versus 26.3 cc, $p < 0.0001$), however, not for the subset of nasopharynx, oral cavity, and other HNC sites (60.4 cc versus 40.1 cc, $p = 0.454$). In contrast, when dichotomized by mean GTV-P (36.6 cc), no differences were noted in minimum, maximum and mean radiation dose to the BP for small and large GTV-P.

Conclusions: In head and neck IMRT, the maximum dose to the BP is primarily affected by nodal GTV. Large GTV-P of the larynx, hypopharynx and oropharynx were associated with a higher maximum BP dose, whereas primary tumors of the nasopharynx, oral cavity and other HNC sites were not.

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Compatibility of the Conditionally-repairable, Multitarget, and Linear-Quadratic Models in Converting Hypofractionated Radiation Doses to Single Doses

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Purpose/Objective(s): Many clinicians use the linear-quadratic (LQ) model in estimating doses of stereotactic radiation therapy, because