MRI Studies of Neuroprotection in a Mouse Model of Radiation Necrosis

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BACKGROUND AND PURPOSE: Radiation necrosis is a severe, but late occurring type of injury to normal tissue, within and surrounding a radiation treatment field that can lead to significant complications for patients with brain tumors. Studies on the characterization of necrosis require the development of robust animal models that will enable a clear histological description of tissue damage in the irradiated brain. Recently, an innovative mouse model of radiation necrosis was developed in our laboratory [**1**] that accurately recapitulates the classic histologic features of radiation necrosis. Figure 1 shows whole-mount (A) and low-power magnification (B, C) histology slides from a mouse brain two months after irradiation. Tissue damage associated with irradiation can be seen clearly in the left hemisphere of the brain. This mouse model can serve as a robust platform for a variety of studies aimed at providing a detailed understanding of the factors that affect the onset and progression of radiation necrosis and the development of neuroprotective agents to reduce or mitigate necrosis. It has been suggested that late time-to-onset radiation necrosis results from acute vascular (endothelial) apoptosis caused by the radiation. The purpose of this study is to test the hypothesis that an inhibitor of GSK-3β (a serine/threonine kinase), SB415286, which protects against endothelial apoptosis [**2**], can reduce radiation necrosis following high-dose radiation treatment.

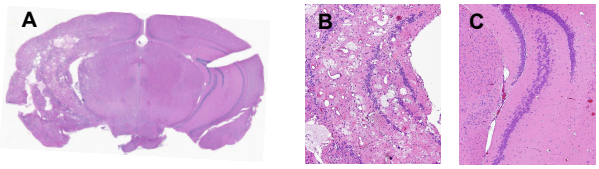

Figure 1. Histology slides of mouse brain 58 days following hemispheric Gamma Knife irradiation (60 Gy). (A) Whole mount, shows clear tissue damage and necrosis involving cortex, hippocampus, and external capsule on the left side of the brain. (right) Low-power magnification, showing radiation damaged (B) and healthy (C) hippocampus (non-irradiated side).

METHODS: Mice were irradiated with the Leksell Gamma Knife Perfexion, a state-of-the-art unit used for stereotactic irradiation of patients with benign and malignant brain tumors. Cohorts of mice administered either the GSK-3β inhibitor SB415286 (1 mg/kg, given 24 hours, 12 hours, and 30 minutes prior to irradiation) or DMSO (control) were exposed to a single 60-Gy dose (50% isodose) of Gamma Knife radiation. Mice were imaged weekly, beginning 5 weeks post irradiation using an Agilent/Varian DirectDrive 4.7-T small-animal MR scanner, equipped with Agilent/Magnex self-shielded gradients and high-performance gradient power amplifiers (International Electric Company). **RESULTS**: Slices from representative T2-weighted spin-echo images collected 7 weeks post-irradiation are shown in Figure 2(A). A region of hyperintensity associated with radiation necrosis is visible in the irradiated control mouse, but is much less evident in the inhibitor-treated irradiated mouse. For each mouse, regions of interest were drawn around the entire brain in 5 contiguous image slices, chosen to include the irradiated region, and histograms of image intensity were constructed, as shown in Figure 2(B). Also shown in this figure is the average image-intensity distribution for a cohort (n=3) of non-irradiated mice. For non-irradiated subjects, the intensity distribution histogram is symmetric; essentially all of the pixels are distributed in the intensity range 0.6 – 1.4 about a normalized mean of 1.0. An intensity threshold of 1.4 (Figure 2(C), arrow) was chosen as the cutoff for normal brain tissue. For images of irradiated mice, the number of pixels exceeding this threshold was taken as the volume of necrosis and was plotted as a function of time post-irradiation. From these plots, the rate of progression of radiation necrosis, derived from the slope of least-squares fits over the time period 5-7 post irradiation, was determined. The mean rate of progression for the irradiated, inhibitor-treated cohort vs. the irradiated, untreated control cohort is shown in Figure 2(C) and demonstrates that that SB415286 treatment reduces radiation necrosis relative to untreated animals.

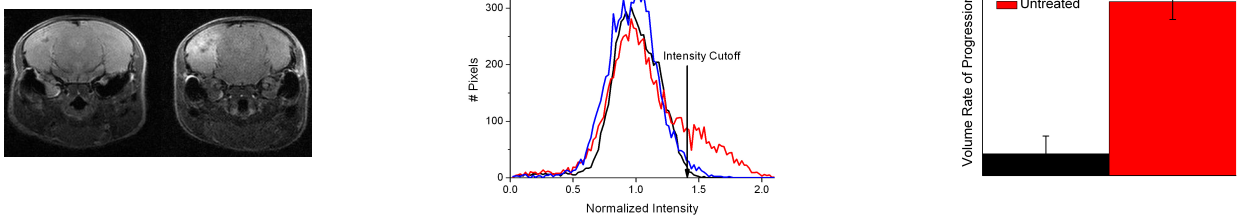

Figure 2. (A) MR images of mouse treated with GSK-3b inhibitor (left) and DMSO (control, right) 5 weeks after GK irradiation. (B) Image intensity histogram for non-irradiated (black); inhibitor-treated, irradiated (blue); and DMSO-treated, irradiated (red); The intensity cutoff used to define hyperintense pixels is indicated. (C) Volumetric rate of radiation necrosis progression, calculated from the slope of number of hyperintense pixels vs. time post-irradiation for the period 5 – 7 weeks.

CONCLUSIONS: Our recently developed mouse model of radiation necrosis provides a platform for understanding the factors that affect the onset and progression of necrosis following irradiation. The data here demonstrate that a GSK-3β inhibitor, SB415286, reduces radiation necrosis relative to untreated, irradiated mice.

REFERENCES: (1) Jost SC, Hope A, et al. A novel murine model for localized radiation necrosis and its characterization using advanced magnetic resonance imaging. *Int J Radiat Oncol Biol Phys* 2009; **75**: 527-33.; **(2)** Thotala DK, Hallahan DE, Yazlovitskaya EM. Inhibition of glycogen synthase kinase 3 beta attenuates neurocognitive dysfunction resulting from cranial irradiation. *Cancer Res* 2008; **68**: 5859-68.