Abstract

While radiotherapy (RT) is fundamental for the treatment of brain tumors, irradiation of the brain frequently causes devastating effects on cognitive function and quality of life. DNA damage within neural stem cells (NSC) is a key factor in the pathogenesis of radiation-induced cognitive dysfunction. The ataxia telangiectasia mutated (ATM) kinase is a central protein in the DNA damage response and a critical determinant of tumor cell survival after radiation. ATM inhibition potently radiosensitizes preclinical models of GBM *in vitro* and *in vivo*. A novel, brain penetrant ATM inhibitor AZD1390, which is predicted to achieve brain tumor concentrations in the range of 1-5nM, is currently in early phase clinical evaluation in combination with RT. In marked contrast to observations in tumor models, genetic knockdown of ATM has radioprotective effects on NSC *in vitro*; the proposed mechanism is via suppression of p53 mediated apoptosis. The purpose of this study was to investigate the impact of AZD1390 on survival responses and mode of death in NSCs exposed to RT *in vitro* and *in vivo*. NSCs were derived from the telencephalon of E13 mouse embryos. Cells were treated with AZD1390 (0.1-10nM) 1 hour prior to ionizing radiation (IR; 0-5 Gy). Mode and timing of cell death was interrogated using IncuCyte live cell analysis to measure proliferation, cytotoxicity and apoptosis up to 72 hours post-IR. Cell viability and neurosphere formation assays were also used to measure radiation sensitivity *in vitro*. C57BL/6 mice received 20Gy hemibrain irradiation +/- 7-day treatment with AZD1390 (10mg/kg). Immunohistochemistry for Ki67 and Sox2 was used to assess effects on NSC in the subventricular zone (SVZ) 50 days post-irradiation. *In vitro* AZD1390 (1-10nM) inhibited ATM kinase function within 1 hour, evidenced by abrogation of KAP1 and p53 phosphorylation. NSCs primarily undergo apoptosis in response to IR. AZD1390 at 1 and 3nM significantly reduced apoptosis in irradiated NSCs (ratios of annexin V area under the curve 1.95 and 2 respectively); 10 nM had no effect on this parameter. Proliferation rates and cell viability after radiation were preserved at all drug concentrations. AZD1390 at 1nM did not modulate radiation effects on neurosphere formation whereas at 10nM a radiosensitizing effect was observed (ratio of SF[3Gy]=0.25). *In vivo*, IR decreased the number of Ki67 positive proliferating cells (92% reduction) and Sox2-positive cells (24% reduction) in the SVZ after 50 days; these effects were not exacerbated by addition of AZD1390. Acute effects (24 hours post-IR) are under investigation. We demonstrate *in vitro* that AZD1390 has radioprotective effects on NSCs at clinically achievable concentrations. *In vivo*, treatment with AZD1390 did not enhance the effects of radiation on NSCs in the SVZ. In the context of its profound radiosensitizing effects on GBM models, the absence of radiosensitization of NSCs both *in vitro* and *in vivo* strengthens the rationale for evaluating AZD1390 in combination with RT in GBM patients.