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Research report

Delayed activation of human microglial cells by high dose ionizing radiation

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ABSTRACT

Recent studies have shown that microglia affects the fate of neural stem cells in response to ionizing radiation, which suggests a role for microglia in radiation-induced degenerative outcomes. We therefore investigated the effects of γ-irradiation on cell survival, proliferation, and activation of microglia and explored associated mechanisms. Specifically, we evaluated cellular and molecular changes associated with exposure of human microglial cells (CHME5) to low and high doses of acute cesium-137 γ rays. Twenty-four hours after irradiation, cell cycle analyses revealed dose-dependent decreases in the fraction of cells in S and G2/M phase, which correlated with significant oxidative stress. By one week after irradiation, 20–30% of the cells exposed to high doses of γ rays underwent apoptosis, which correlated with significant concomitant decrease in metabolic activity as assessed by the MTT assay, and microglial activation as judged by both morphological changes and increased expression of Glut-5 and CR43. These changes were associated with increases in the mRNA levels for IL-1α, IL-10 and TNFα. Together, the results show that human CHME5 microglia are relatively resistant to low and moderate doses of γ rays, but are sensitive to acute high doses, and that CHME5 cells are a useful tool for in vitro study of human microglia.

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1. Introduction

The loss of neurogenesis after cranial radiotherapy has been associated with delayed cognitive decline in long-term survivor of malignant brain tumors, particularly in children (Anderson et al., 2000; Lee et al., 1989; Nagel et al., 2006). Likely, these effects may result from delayed cell death of irradiated cells and impairment in the differentiation potential of neural stem cells.

Microglial response to irradiation has emerged as a major mechanism underlying health hazards associated with radiotherapy, such as cognitive deficit. In response to exposure to ionizing radiation (IR), activated microglia have been shown, in vitro, to induce the proliferation of neural stem cells and promote the differentiation of neurosphere-derived cells (Deierborg et al., 2010). A recent study showed that microglial responses might improve the recovery of neural stem cells after irradiation (Hellstrom et al., 2011). However, several other studies demonstrated that activated microglia inhibit hippocampal neurogenesis and induces spatial memory dysfunction (Jenrow et al., 2013; Monje et al., 2002), which may be due to IR-induced rapid microglial inflammatory response (Ekdahl et al., 2009; Kalm et al., 2009a; Monje et al., 2003). Therefore, further understanding of the cellular mechanisms of microglial response to IR is essential to better characterize neuroinflammation after brain irradiation. However, the lack of independent phenotypic markers that distinguish microglia from peripheral macrophages, and the obstacle to obtain primary human microglia make it difficult to characterize the activation of microglia (Santambrogio et al., 2001; Ulvestad et al., 1994). This study used a well-characterized human microglial cell line CHME 5 (Janabi et al., 1995) to investigate the direct effects of low and high doses of γ rays on cell survival, proliferation, oxidative stress, and inflammatory responses.

2. Results

2.1. Irradiation decreases microglial metabolic cell survival

The metabolic survival of CHME5 microglial cells in response to irradiation with ^{137}Cs γ rays was studied with the MTT assay. MTT is a colorimetric assay for measurement of mitochondrial activity through the reduction of yellow MTT by mitochondrial succinate

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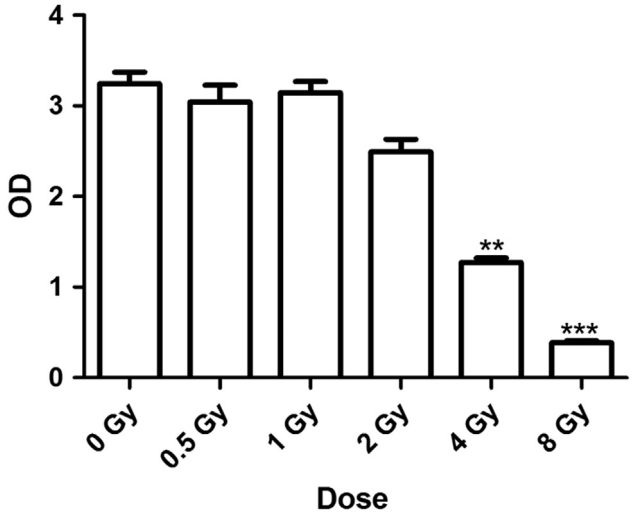

Fig. 1. The effect of γ-irradiation on mitochondrial metabolic activity of microglia. Human microglial CHME-5 cells were exposed to ^{137}Cs γ rays to acute mean absorbed doses in the range of 0.5–8 Gy, and metabolic survival was determined at 7 days following irradiation by the MTT colorimetric assay. High dose irradiation significantly decreased the metabolic survival of CHME5 microglial cells. Data from three independent experiments are represented as Mean ± SEM followed by Dunn's Multiple Comparisons Test (**$p < 0.01$;***$p < 0.001$ vs. 0 Gy).

dehydrogenase (complex II). As shown in Fig. 1, there was a dose-dependent decrease in the metabolic activity of microglia. Although a small reduction in MTT signal was observed at 7 days following exposure to 2 Gy of ^{137}Cs γ rays, a significant decrease was only observed in cells exposed to IR at doses greater than 2 Gy. About 50% percent reduction in cell survival was observed at 7 days after exposure to 4 Gy, and a greatly enhanced reduction after exposure to 8 Gy.

2.2. Irradiation induces oxidative stress in microglia

In this set of studies we investigated the effects of irradiation on general cellular reactive oxygen species (ROS). After 1 h of exposure to IR, CHME 5 cells were stained with DCFDA to evaluate total ROS (Fig. 2(A)). Both low (0.5 Gy) and high dose (8 Gy) γ rays caused a significant increase in cellular ROS. In parallel studies, we also measured mitochondrial superoxide generation in live CHME 5 cells before and after exposure to γ rays. The results (Fig. 2(B)) indicated that only high dose (8 Gy) γ radiation induced significant upregulation in mitochondrial superoxide level after 15 min of exposure, suggesting that an early and selective impairment in mitochondrial function occurred in 8 Gy-exposed CHME 5 cells.

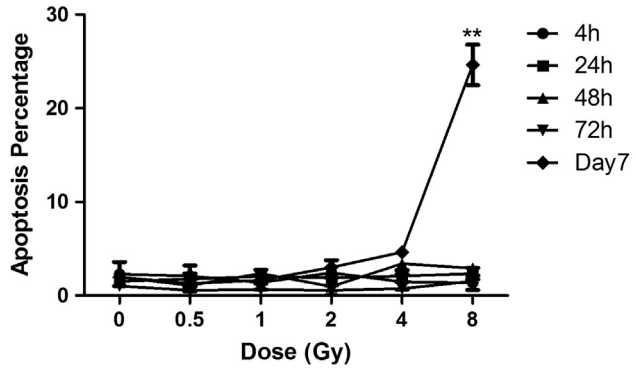

Fig. 3. High dose irradiation induces apoptosis in microglia. Human microglial CHME-5 cells were exposed to ^{137}Cs γ rays (2–8 Gy) and apoptosis was determined over a week period of time following the radiation exposure by FITC-Annexin V labeling. Data from three independent experiments are represented as Mean ± SEM and followed by Dunnett Multiple Comparisons Test (**$p < 0.01$ vs. 0 Gy).

2.3. Irradiation induces apoptosis in microglia cells

The effect of radiation on apoptosis of microglial cells was studied using the FITC Annexin V detection reagent. The cells were analyzed between 4 h and 7 days following exposure to IR. No significant apoptosis was detected after exposure to γ-ray doses in the range of 0.5–8 Gy at 4 h, 24 h, 48 h or 72 h. However, 20–30% of CHME 5 microglial cells exhibited apoptosis at 1 week after exposure to 8 Gy (Fig. 3).

2.4. Irradiation causes G2/M arrest in microglia

We examined the effects of IR on the progression through the cell cycle of CHME 5 microglial cells at 24 h, 48 h and 7 days after exposure to high and low dose γ rays (Fig. 4). Irradiation with 8 Gy caused reduction in the fraction of cells in S phase as well as G2/M arrest at 24 h after exposure. However, 48 h after exposure, the effect of irradiation on cell cycle was attenuated and only a mild increase in the sub-G1 peak was observed 7 days after exposure (Fig. 4).

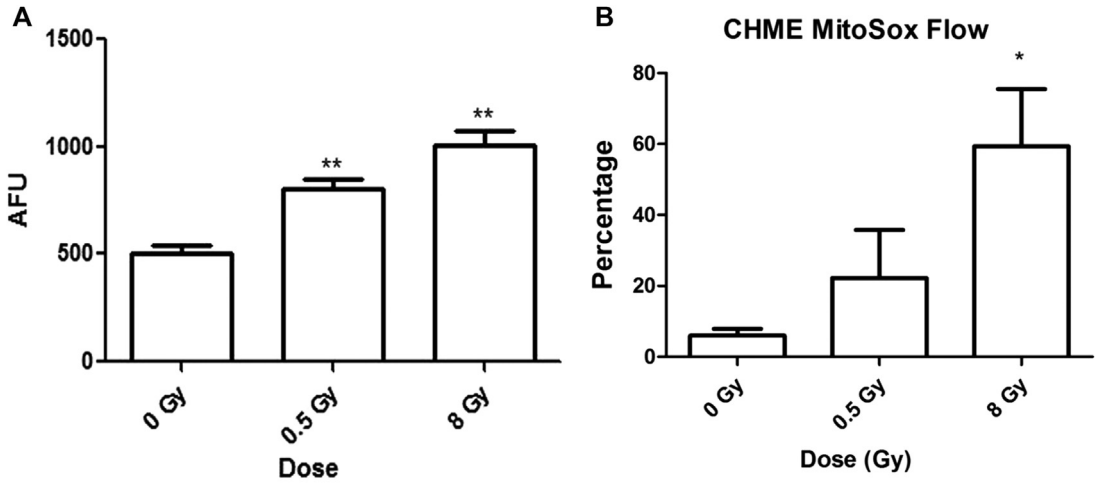

Fig. 2. Ionizing radiation induces oxidative stress in microglia. A. Human microglial CHME-5 cells were exposed to acute 0.5 or 8 Gy of ^{137}Cs γ rays and reactive oxygen species (ROS) in CHME5 cells was determined by DCFDA staining 1 h following the radiation exposure. B. Human microglial CHME-5 cells were exposed to acute 0.5 or 8 Gy of ^{137}Cs γ rays and mitochondrial superoxide anions were measured by Mitosox Red staining 15 min following irradiation. Data from four independent experiments are represented as Mean ± SEM followed by Dunn's Multiple Comparisons Test (*$p < 0.05$;**$p < 0.01$ vs. 0 Gy).

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