



Inhibition of Caspase-Dependent Apoptosis by Inactivating the iNOS Pathway Protects Human T Cells against Gamma Radiation Injury

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Exposure to ionizing radiation results in DNA breaks that activate the ATM and CHK2 pathways. NF- κ B/p53/CDC25 are then activated, which arrests the cell cycle (Houtgraaf et al., 2006). This in turn leads to acute radiation syndrome (ARS) followed by multiple organ dysfunction syndrome (MODS) and multiple organ failure (MOF). iNOS expression and NO production increase after radiation exposure (Inano and Onoda, 2005; Zhong et al., 2004; Chang et al., 2003; Chung et al., 2003). We previously showed that inhibiting iNOS expression prevents injury induced by hemorrhage (Kiang et al., 2004, 2006, 2007a, 2007b) and hypoxia (Kiang et al., 2008). We, therefore, investigated whether gamma radiation-induced activation of the iNOS pathway is associated with increased caspase activity and apoptosis. Furthermore, we studied whether agents inactivating the iNOS pathway limited the caspase-dependent apoptosis induced by gamma radiation.

Human Jurkat T cells were exposed to gamma radiation (4 Gy). The irradiated cells were collected at different times after irradiation. In these cells we measured cell viability first. Then, cell lysates were prepared to measure protein levels of KLF6, KLF4, NF- κ B, iNOS, p53, Bcl-2, and Bax, NO production, lipid peroxidation, apoptosome formed by cytochrome c, caspase-9, and Apaf-1, and caspase-3 enzymatic activity. To evaluate the relationship between iNOS and the caspases, we inhibited iNOS expression by treating cells with the iNOS inhibitor 17-DMAG or iNOS siRNA.

Gamma radiation exposure increased iNOS expression by increasing levels of its transcription factors, NF- κ B-p50 and KLF6 within 4 hr after irradiation. Twenty-four hours after irradiation, cell viability was reduced by 35%. In these cells, nitrate (representing NO production) and MDA (representing lipid peroxidation) increased in a radiation dose-dependent and post-radiation time-dependent manner. Apoptosome formation (complex of caspase-9, cytochrome c, and Apaf-1, Jiang and Wang, 2004, Kiang, 2006) and caspase-3 enzymatic activity were significantly elevated, suggesting gamma radiation-induced apoptosis is mediated by the intrinsic caspase-dependent pathway. Treatment with iNOS inhibitor 17-DMAG 24 hr prior to gamma radiation significantly limited these biomolecular changes and increased the cell viability. Treatment with iNOS siRNA to silence the iNOS gene produced similar results, further confirming the correlation between the iNOS pathway and the radiation-induced apoptosis.

These results suggest gamma radiation activates the iNOS pathway, which leads to caspase-3-dependent apoptosis. NO, MDA, and caspase-3 are potential biomolecules responding to irradiation. Agents including 17-DMAG that inhibit the iNOS pathway may prove useful for treating radiation injury.

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