154 Ionizing Radiation Activates Caspase-8/FLICE in Mouse Embryo Fibroblasts with A Targeted Disruption of The Insulin-like Growth Factor I Receptor Gene

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We previously reported that the insulin-like growth factor I receptor (IGF-IR) inhibits ionizing radiation(IR)-induced cell death by employing R- and R+ cells, which are mouse embryo fibroblasts with a targeted disruption of the IGF-IR gene and a transfectant overexpressing the human IGF-IR, respectively. To approach the molecular mechanism, a possible cross talk with the death receptor pathway was examined using the same cell lines. Caspase-8/FLICE is well known to be activated through death receptors. Caspase-8 activities were significantly increased only in R- cells in a dose-dependent manner 48 h after X-irradiation, but not in R+ cells. To see the involvement of death receptor activation, expression of Fas and Fas-L was monitored by a flow cytometry. Significant increase in their expression was detected in neither of the cell lines following irradiation. Interestingly, direct activation of Fas or TNF-R1 by anti-Fas antibody or TNF-α induced similar increase in caspase-8 activities in the both cell lines. Collectively, these results imply that IR may induce caspase-8 activation in a death receptor-independent manner and the IGF-IR could inhibit the unique pathway at least in these cell lines.

155 Mechanism of Cytochrome c Release in Radiation-induced Apoptosis: Roles of Bax, VDAC, ROS and $\Delta\Psi$m.

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A main mechanism of the cytochrome c (Cyt-c) release from mitochondria (MT) downstream of the p53-dependent Bax up-regulation and Bcl-2 down-regulation after X-ray irradiation was investigated using intracellular MT and isolated MT particles. p53 protein accumulated and was phosphorylated on Ser-15 with time after 5 Gy. By its transregulation, Bcl-2 and Bax were repressed and enhanced respectively, to reach the Bax/Bcl-2 ratio to ~7 at 6 h. Cytosolic Bax was moved rapidly and time-dependently to MT, with reciprocal decrease in Bcl-2 on MT. The Cyt-c release from MT to S100 increased with time after irradiation, concurrent with Bax increase. Bax interacted with VDAC in good correlation with Bax translocation and Cyt-c release after X-ray stimulus, suggesting a possibility that Cyt-c may pass through the Bax/VDAC channel on the outer MT membrane during the early phase. The Rh123 and DHE flow cytometry revealed that swelling, superoxide (ROS) production and $\Delta\Psi$m reduction in cellular and isolated MT occurred during a late phase after considerable loss of Cyt-c that injured severely the electron transport chain.

156 H_2O_2-Induced Activation of SAPK/JNK Regulated by Phosphatidylinositol 3-Kinase in Chinese Hamster V79 Cells

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To clarify activation mechanisms of stress-activated protein kinases/c-Jun N-terminal kinase (SAPK/JNK) during oxidative stress, the roles of phosphatidylinositol 3-kinase (PI 3-kinase), concentration of intracellular calcium ($[Ca^{2+}]_i$), in hydrogen peroxide (H_2O_2)-induced SAPK/JNK activation were examined in Chinese hamster V79 cells. SAPK/JNK was dose-dependently activated after H_2O_2 treatment (from 10 μM to 10 mM) and a PI 3-kinase inhibitor (wortmaninn), intracellular calcium chelator (BAPTA-AM) inhibited this activation. An increase in $[Ca^{2+}]_i$ was observed after treatment with H_2O_2. Immunoprecipitation revealed that a PI 3-kinase regulatory subunit, p85α was associated with insulin receptor substance 1 (IRS-1) phosphorylated by H_2O_2 treatment. Furthermore, the formation of this complex of p85 α and phospho-IRS-1 was abolished by the presence of BAPTA-AM. These results indicated that the PI 3-kinase activated through phosphorylation of IRS-1 upstream of SAPK/JNK after H_2O_2 treatment of V79 cells and that $[Ca^{2+}]_i$ was a regulation factor for phosphorylation of IRS-1.