101 Sensitizing Mechanism of Heat-induced Apoptosis by Nitroxide Tempo

Qing-Li ZHAO[1], Yoshisada FUJIWARA[1], Ryohei OGAWA[1], Takashi KONDO[1], [1]Dept. Radiol. Sci. Toyama Medical and Pharmaceutical Univ.

Heating induces apoptosis as well as several cellular responses. Tempo has been shown to exert the SOD-like activity against ROS, but to activate the JNK signaling pathway for induction of apoptosis. Here we describe a drastically sensitizing effect of Tempo on heat-induced apoptosis. We used either a single or combination application of Tempo (1–10 mM) and/or 44°C heating (10–30 min) to U937 leukemia cells and analyzed apoptosis, mitochondrial transmembrane potential and amount of superoxide by means of flow cytometry. The combined treatment with 44°C and 1–10 mM Tempo for 30 min induced a high saturating level of 95–100% cell death detected 6 h after treatment, whereas 1–5 mM Tempo alone had no effect at 37 degrees Celsius. A10 min-combined treatment with 44°C-plus-5 mM Tempo enhanced apoptosis in a time-dependent manner, through a mechanism to produce low-mitochondrial transmembrane potential cells and low-superoxide cells. However, neither 44°C heating for 10 min nor treatment 5 mM Tempo at 37°C for 10 min had no such effects. zVAD-fmk suppressed the sensitization. Thus, the optimal concentration of Tempo sensitizes U937 cells to the heat-induced apoptosis through mitochondria-mediated process.

102 Possible involvement of *leu*-13 gene expression in interferon-induced resistance in human cells to X-ray cell-killing

Kazuko KITA[1], Yu-Ping WU[1], Shigeru SUGAYA[1], Nobuo SUZUKI[1], [1]Dept. Environment. Biochem. Graduate Sch. Med. Chiba Univ.

We previously reported human interferon (HuIFN)-induced resistance of human cells to X-ray cell killing and mutagenicity. In the present study, we investigated genes whose expression was altered in human RSa cells treated with HuIFN, using a PCR based mRNA differential display and Northern blotting analysis. RSa cells were established from human fetal fibroblasts with high sensitivity to cell-killing induced by X-ray. Messenger RNA expression levels for Leu-13 (a leukocyte antigen) cell surface protein were markedly up-regulated in RSa cells after IFN treatment. In contrast, the Leu-13 mRNA levels were constitutively higher in X-ray-resistant human IF^r-2 and F-IF^r cells than in RSa cells. IF^{r}2 and F-IF^r cells were strains derived from RSa cells. Furthermore, pretreatement of RSa cells with antisense oligonucleotides for Leu-13 mRNA resulted in an increased susceptibility to X-ray cell-killing. These results suggest that Leu-13 is involved in HuIFN-induced X-ray resistance in human cells.

103 Mechanisms Involved in the Expression of p53-dependent Apoptosis Induced by X-rays

Hisako NAKANO[1], Hiromichi YONEKAWA[1], Kunio SHINOHARA[2], [1]Dept. of Lab.Animal Sci.,Tokyo Metropolitan Inst. Med. Sci. [2]Grad. Sch. Med., Univ. of Tokyo

Human leukemic MOLT-4 cells undergo apoptosis after X-irradiation through p53-dependent pathway. In MOLT-4 cells, p53 (wild type) is stabilized and increased after X-irradiation. Western blotting analysis using antibodies for phosphorylated p53 at serine 15 or serine 20 showed that X-irradiation induced the increase in phosphorylated p53 at serine 15, but not in the one at serine 20. Colony survival and cell death (apoptosis) by the dye-exclusion test were compared in a stable clone transfected with mutant p53 cDNA (B3). Development of apoptosis was inhibited almost completely at 24 hr of post-irradiation and started later, while decrease in colony survival after X-irradiation was inhibited partially. These results suggest that in MOLT-4 cells, p53 is stabilized by the phosphorylation at serine 15 and that the expression of apoptosis in B3 is simply delayed.