130 Analysis of the Upstream Region of the Radiation-induced Genes for the Immediate-early Induction by the Modified Reporter Assay.

Hong WAN, Izumi TANAKA and Hiroshi ISHIHARA*;
Bioregul. Res. Group, Natl. Inst. Radiol. Sci., Chiba 263

We found that interleukin(IL-1)-β gene which encodes a radioprotector is transcriptionally stimulated immediately after x-irradiation in murine monocytic cells, and that sequence-specific binding activities of nuclear proteins to the upstream region of the IL-1β gene are modified immediately after irradiation in the monocytic cells. To analyze the contribution of the binding to the gene expression, the modified reporter assay was developed.

Reporter genes directed by the upstream region of the radiation-responsive genes including the IL-1β gene were constructed and introduced to a macrophage cell line. We found the accumulation of the transcripts of the reporter genes were observed immediately after the induction. The function of the nucleotide sequences for the immediate-early induction by radiation can be estimated by the method.

131 Cellular response and p53 accumulation following heat shock in normal human cells

Mana MIYAKODA, Keiji SUZUKI, Seiji KODAMA and Masami WATANABE; Div. Radiate.& Life Sci., Nagasaki University, Nagasaki 852

p53 protein is accumulated following exposure to various stresses and it transactivates gene for p21, GADD45, BAX and Bcl-2 proteins. The induced proteins are hypothesized to associated with the regulation of cell cycle at G_1/S and apoptosis. We examined here the accumulation of p53 protein by heat-shock treatment in normal human embryonic cells. Cells were heat shocked at 43°C for 2 hours and recovered at 37°C subsequently. The accumulation p53 protein was measured by western blotting. The maximum level of p53, and p21 and GADD45 was observed 3 and 4 hours after the heat shock, respectively. The fraction of S-phase gradually decreased 4 hours after the heat shock, and G_1 and G_2 arrest were evident after 12 hours. These results suggest that p21 and GADD45 proteins induced by p53 were involved in these cell cycle regulation.

132 Activation of dual signal transduction pathways and p53 protein accumulation in normal human embryonic cells irradiated with X-rays

Keiji SUZUKI, Seiji KODAMA, and Masami WATANABE
Div. Radiat. & Life Sci., Nagasaki University, Nagasaki 852.

p53 protein is a nuclear phosphoprotein, whose level was accumulated and activated by various cellular stresses, such as ionizing radiation, UV-irradiation, and heat shock treatment. Recently, we have reported that wortmannin, an inhibitor for protein kinase, inhibits p53 accumulation, suggesting that activation of signal transduction pathway phosphorylating p53 protein is required for its activation. In order to determine the signal transduction pathway(s) involving an accumulation of p53 protein, we examined the signals transduced from the nucleus and the plasma membrane. When blunt-ended restriction enzyme, Pvu II, was introduced by permiabilization of cells, p53 protein was accumulated, and this activation was suppressed by concomitant treatment of wortmannin. In contrast, inhibition of glycolipids synthesis, by treatment of cells with DL-threo-PDMP, also diminished p53 accumulation by X-ray irradiation. These results indicate that both nucler-originated and membrane-originated signals are responsible for the regulation of the p53 function in X-ray-irradiated normal human cells.