245 Irradiation reduces levels of myeloperoxidase mRNA through TNF production in HL60 cells

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Myeloperoxidase(MPO) is a heme containing glycoprotein present in the primary granules of mainly polymorphonuclear leukocytes. This enzyme reacts with H_2O_2 and halide ion as part of the microbicidal system, and is involved in the modulation of inflammatory responses. We examined the regulation of MPO gene by irradiation in human promyelocytic cells HL60. We found that irradiation reduced expression of MPO mRNA at a dose- and time-dependent manner. The decreased levels of MPO mRNA after irradiation were also observed in THP1 monocytic cells. Irradiation stimulated tumor necrosis factor α (TNF-α) production in these cells and exogenously added-TNF decreased the levels of MPO transcripts. Moreover, treatment of cells with anti-TNFα antibody inhibited the decrease in MPO mRNA levels by irradiation. We also found that irradiation reduced transcriptional rate of MPO gene and stability of MPO mRNA in these cells. Our results suggest that irradiation reduces the steady-state levels of MPO mRNA through production of TNFα in HL60 cells and that regulation of MPO gene by irradiation occurs at both transcriptional and post-transcriptional levels.

246 Introduction of human gadd45 gene to human immortal cell lines using the inducible expression vector

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Gadd genes are induced by a variety of DNA-damaging agents and growth arrest treatments. It is hypothesized that gadd45 gene product is involved in p53-dependent cell cycle checkpoint after irradiation. To know the role of GADD45 in radiation-induced G_1 arrest, we isolated cDNA for human GADD45 and constructed an expression vector containing human gadd45 gene using the modified *E. coli* lactose operon system for the control of gene expression. SV40-transformed GM638 and AT5BIVA cells were transfected with the lac repressor gene and several colonies expressing the lac repressor were isolated. Sense- and antisense-human gadd45 genes were then transfected into lac repressor positive cells to investigate the stable induction of the gadd45 gene by the IPTG inducer.

247 The MAPK family is not activated by X-ray irradiation

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There is now a growing evidence indicating that distinct subtypes of MAP kinase are activated in response to growth stimulation and several cellular stress. Cultured mouse m5S cells in quiescent stage can be activated to express c-jun, enter S phase and proceed to mitosis in response to X-ray irradiation at doses as 2 cGy. Here, we studied the signal amplification pathway in the mouse m5S cells, and found that X-ray irradiation did not activate ERKs, JNK/SAPKs and p38 while TPA strongly activated ERKs but not JNK/SAPKs, and UV irradiation activated JNK/SAPKs but not ERKs. These observations suggest that X-ray irradiation mediates signaling pathway which is distinct from UV and TPA, but these related to the transcriptional activation.