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induced by X irradiation also participated in the ROS production.

In conclusion, the present study demonstrated that the ROS generated by NAD (P) H oxidase was partly responsible for the caspase 3-dependent apoptosis. Since this apoptosis was partly inhibited by antioxidants like PBN and trolox, the post-irradiation treatment with antioxidants may be used as a new remedy for radioprotection.

Enhancement of radiation-induced apoptosis in HL 60 and MOLT-4 cells by oxygen and hypoxic radiosensitizer etanidazole

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The distribution of hypoxic cells in the tumor is thought to be critical against a cure rate of radiation therapy, because ionizing radiation induces less efficiently cell death under the hypoxic conditions than under the aerobic condition. The killing effect of ionizing radiation on cultured mammalian cells was usually judged by the loss of clonogenic ability. Recent studies showed that two types of cell death, necrosis and apoptosis, were main components of the loss of clonogenic ability. However, there are little reports about oxygen effects on radiation-induced apoptotic cell death. The present study was carried out to examine how the hypoxia influenced the ionizing irradiation-induced apoptosis in cultured mammalian cells with the aid of a specially designed gas-exchangeable chamber. Furthermore, we examined whether the hypoxic cell radiosensitizer, etanidazole, sensitized the apoptotic cell death under the hypoxic condition.

Two cell-lines derived from human lymphocytes, HL 60 and MOLT-4, were employed and exposed to 15 Gy of X-rays under the aerobic and hypoxic conditions. In the case of experiments with etanidazole, both were treated with 10 mM etanidazole for 90 min before exposure to X-rays. The apoptotic morphological changes of nuclei and induction of ladder-like DNA fragmentation were accessed by fluorescence microscopy and agarose gel electrophoresis, respectively. In HL 60 cells, the results showed that the apototic cell death and the activation of caspase 8, 9 and 3 were less induced in the hypoxic cells than in the aerobic ones. Furthermore, treatments of the cells with etanidazole enhanced radiation-induced apoptosis as well as the activation of caspase family under the hypoxic condition. However, in MOLT-4 cells, hypoxia did not reduce radiation-induced apoptotic cells and the activation of caspase family. The treatment with etanidazole did not affect the induction of radiation-induced apoptosis under the hypoxic condition. To investigate the relationship between the radiation-induced DNA double-strand breaks (dsb) and the induction of apoptosis, the dsb were measured by pulsed-field gel electrophoresis immediately after X irradiation. In both cell lines, the radiation-induced dsb in the hypoxic condition was sig-

nificantly smaller than that in the aerobic condition, and treatments of cells with etanidazole enhanced the radiation-induced dsb under the hypoxic condition. Moreover, in 5-bromo-2'deoxyuridine-incorporated cells, the remarkable sensitization of radiation-induced apoptosis was observed in HL 60 and not in MOLT-4 cells.

These results indicated that the radiation-induced apoptosis of HL 60 cells was initiated by DNA dsb and treatment with etanidazole sensitized apoptosis through the enhancement of dsb induction in the hypoxic condition, but the radiation-induced apoptosis of MOLT-4 cells occurred through damage other than DNA, i. e., lipid and/or protein oxidations. It seems that the incidence of oxygen effects and sensitizing effects of etanidazole on radiation-induced apoptosis are dependent on the difference of radiation targets.

The effect of estrogen receptor on the induction of cytochrome P 450 mRNA via aryl hydrocarbon receptor

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Aryl hydrocarbon (Ah) receptor agonists, such as benzo(a)pyrene (BP) and 2,3,7,8-tetrachrolodibenzo-p-dioxin (TCDD) are known to exert the effects of endocrine disruption, and to induce certain kinds of cytochrome P 450 (CYP) as well as phase II enzymes. BP induced CYP 1 A 1 and CYP 1 B 1 mRNA in human breast cancer MCF-7 cells in dose dependent manner, but co-application of estradiol 17β (E 2) reduced CYP 1 A 1 induction with no effect on CYP 1 B 1 induction. In human hepatocyte HepG 2 cells containing no functional estrogen receptor, this reduction was not detected. These results suggested a possibility that the reduction was due to estrogen receptor activation. To elucidate the interaction between Ah receptor and estrogen receptor, I examined the effect of growth factors whose expression or sensitivity depended on estrogen receptor activation on CYP 1 A 1 induction in MCF-7 cells. Epidermal growth factor and insulin like growth factor-I did not alter CYP 1 A 1 mRNA induction by BP. DNA mobility shift assay showed that E 2 had no effect on Ah receptor binding to xenobiotic responsive element, Ah receptor/Arnt heterodimer binding region in upstream sequence of CYP 1 A 1 and CYP 1 B 1. AIB 1, a transcriptional factor overexpressed in breast cancer, was transfected into HepG 2 cells. AIB 1 transfected HepG 2 cells reduced CYP 1 A 1 induction by BP. These results suggest that AIB 1 may involve Ah receptor mediated gene transcription and interaction with estrogen receptor.