203 Apoptosis Induction in Gastric Adenocarcinoma MKN45 Cells Exposed to X-Rays in the Presence of 1-(3-C-Ethynyl-β-D-*Ribo*-Pentofuranosyl) Cytosine (ECyd)

Akiko IWAHARA[1], Osamu INANAMI[1], Kenji TAKAHASHI[1], Yasuhiro KON[2], Akira MATSUDA[3], Mikinori KUWABARA[1], [1]Lab. Radiat. Biol. Grad. Sch. Vet. Med. Hokkaido Univ. [2]Exp. Animal Sci. Grad. Sch. Vet. Med. Hokkaido Univ. [3]Grad. Sch. Pharm. Sci. Hokkaido Univ.

1-(3-C-Ethynyl-β-D-*ribo*-pentofuranosyl) cytosine (ECyd) was developped as an anticancer drug to induce apoptosis. In this study, we examined whether the exposure of MKN45 cells to X-rays in the presence of ECyd at the low concentrations, which induces no apoptosis itself, induced apototic cell death. MKN-45 cells were treated with 100 nM ECyd 1 hour before irradiation. After irradiation with 20 Gy, apoptosis was morphologically evaluated by fluorescence microscopy using propidium iodide staining as well as electron microscopy. When cells were incubated for 36 h after X irradiation or after the treatment with ECyd, only 5% of apoptotic cells appeared. However, when cells were irradiated with X-rays in the presence of ECyd, about 25% of total cells was apoptosis. This apoptosis was significantly reduced by the treatment with TPCK or Z-VAD-fmk. These results suggested that chymotrypsin-like and caspase-like proteases were responsible for apoptosis induced by co-treatment of X-rays with ECyd.

204 Enhancement of X-Ray-Induced Cell Death in Chinese Hamster V79 Cells by ECyd

Daisuke IIZUKA[1], Osamu INANAMI[1], Kenji TAKAHASHI[1], Akira MATSUDA[2], Mikinori KUWABARA[1], [1]Lab. Radiat. Biol. Grad. Sch. Vet. Med. Hokkaido Univ. [2]Grad. Sch. Pharm. Sci. Hokkaido Univ.

1-(3-C-ethynyl-β-D-*ribo*-pentofuranosyl)cytosine (ECyd) is a newly developed anti-tumor drug. Recently, we revealed that the X-ray-induced apoptosis was enhanced by low doses of ECyd. This study was performed to examine whether ECyd sensitized X-ray-induced cell death in clonogenic assay. V79 cells were treated with 1 μM ECyd 1 hour before X irradiation. Survival fractions were measured using a standard colony formation assay. For measurements of apoptosis, the apoptotic morphological changes of nuclei were accessed by fluorescence microscopy. The clonogenic assay showed that ECyd significantly enhanced X-ray-induced cell death and the microscopic observation revealed that the apoptotic induction was also enhanced by co-treatment of X-rays with ECyd. This apoptosis induced by X-rays and ECyd was significantly decreased by the treatment with Ac-DEVD-CHO (a caspase-3 inhibitor). These results indicated that ECyd was able to sensitize X-ray-induced cell death through caspase-3-dependent apoptosis.

205 Suppressive Effect of p53 on Heat-induced Multiple Centrosomes

Mana MIYAKODA[1], Keiji SUZUKI[1], Seiji KODAMA[1], Masami WATANEBE[1], [1]Lab. Radiat. Life Sci., Sch. Pharm. Sci., Nagasaki Univ.

Activation of p53 suppresses genome destabilization by DNA-damaging agents. Heat shock, which does not induce DNA damage but protein denaturation, also activates p53. This suggests a possibility that p53 protein inhibits heat-induced genome instability. We first examined whether heat damages centrosomes in p53-functional cells (normal human diploid HE49, HT1080, MCF7) and non-functional cells (T24, RD, A431, H1299). Heat shock caused dispersed or denatured centrosomes, and multiple centrosomes were detected in all cells 24 hr after heat shock. Then growth of cells with multiple centrosomes was suppressed in HE49, HT1080 and MCF7 cells. While the number of chromosomes changed after heat shock, it was less significant in cells with the functional p53. These results suggest that p53 inhibits the growth of cells with multiple centrosomes, which result in numerical changes of chromosomes.