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4 and 8 weeks after infection in the inoculated suckling mice, but in only 4 weeks after infection in the inoculated adult mice. The detection period of the IgM antibody by both mice may be different because the suckling mice were caused persistent infection by the virus and were continuously stimulated by viral antigens.

3. Con A responses of splenic cells from the suckling BALB/c mice which persistently infected confidently reduced as compared with uninfected BALB/c mice. In contrast, Con A responses of splenic cells from the adult mice which transiently infected were similar to uninfected BALB/c mice. These results indicated that cellular immune response was important to eliminate hantavirus

from mice.

4. P388D1 cells which were inoculated hantavirus by antidody-dependent enhancement were infectious rate of only 1 to 2% at first, but until 12 days post inoculation viral antigens were able to detected almost 100% of cells. Further, these cells could be cultured for a long time. Then, P388D1 cells inoculated hantavirus may be useful to measure the cytotoxic activity to viral antigen.

5. Nucleocapsid protein gene of hantavirus inserted into eukaryotic expression vector was transfected to P815 cell. As a result, mRNA of Nucleocapsid protein was detected in some of clone.

Apoptosis and intracellular signal transduction induced in X-irradiated MOLT-4 cells

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Ionizing radiation is widely utilized for a tumor therapy. Recently, it has been reported that ionizing radiation as well as Fas-antigen, NGF, anticancer drug, UV and reactive oxygen species induce apoptotic cell death in various tumor cells. The signal transduction leading to apoptosis was wellknown to be related to the *p53* tumor suppressor gene. However, many tumors have been reported to exhibit unfunctional *p53* mutations. Therefore, considering the radiation treatment of *p53*-independent tumors, it is important to study the mechanism of radiation-induced *p53*-independent apoptosis. This study was performed to clarify the relationship between the activation of *p53*-independent signal pathway (the increase of intracellular calcium ion $[Ca^{2+}]_i$, the activation of stress-activated protein kinase/

c-Jun N-terminal kinase (SAPK/JNK) and interleukin-1 β -converting enzyme (caspase)) and apoptosis in X-irradiated human leukemia cell line, MOLT-4.

First, to check whether *p53* accumulated in X-irradiated MOLT-4 was functional, the cell-cycle distribution of MOLT-4 was examined after X irradiation by flowcytometry. No *p53*-dependent G_1 arrest was observed after X irradiation, but G_2 arrest and sub- G_1 fraction showing apoptosis were detected. This result indicated that *p53* induced in X-irradiated MOLT-4 cells failed to function well, and, therefore, X-ray-induced apoptosis occurred by the *p53*-independent manner. As a *p53*-independent pathway to apoptosis, the ceramide-SAPK/JNK-caspase pathway has been shown in the NGF- or

anticancer drug-induced apoptosis. In MOLT-4 cells exposed to 15Gy of X-ray, DNA fragments were detected by agarose gel electrophoresis 12 h after X irradiation. This DNA fragmentation was inhibited by not only a metabolic product of ceramide, sphingosine-1-phosphate, but also a specific inhibitor of caspase-3 (Ac-DEVD-CHO). Western blot analysis showed that the phosphorylated active form of SAPK/JNK appeared 1 h after X irradiation and its concentration was maintained at the constant level for at least 9 h. The active form of caspase-3 (p20) was detected 3 h after X irradiation and the subsequent cleavage of nuclear enzyme poly (ADP-ribose) polymerase by p20 were detected. Furth-

ermore, a cell permeant $[Ca^{2+}]_i$ chelator, (acetyloxymethyl-) 1, 2-bis (*o*-aminophenoxy) ethane-*N, N, N', N'*-tetraacetic acid, gave the lag in the onset of the activation of SAPK/JNK and caspase-3 as well as the DNA fragmentation, but this compound gave no effect on the X-ray-induced accumulation of aberrant p53.

These results obtained from p53-unfunctional MOLT-4 cells demonstrated that X irradiation induced the increase of $[Ca^{2+}]_i$ which became a trigger of the activation of SAPK/JNK and caspase-3 and produced the ladder-like DNA fragments. These data may provide useful information about the radiation treatment of p53-unfunctional tumors.

Characterization of Liver Cytochrome P450 Subfamilies in Seals from the Coast of Hokkaido and Factors Responsible for Their Activities and Contents

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In order to understand species-specific characterization of liver microsomal cytochrome P450 subfamilies in two species of seals, *Phoca largha* and *Phoca fasciata*, and to elucidate factors responsible for their enzymatic activities and contents, the P450 spectrum, P450-dependent enzyme activity and Western blotting analysis using fresh livers of seals were studied. The results and discussion in this study can be summarized as follows. (1) Dithionite difference spectra of CO-treated seal microsomes showed two peaks around 448nm and 420nm. Appearance of the peak at around 420nm indicates that sample degradation occurred during storage conditions prior to microsome preparation. (2) Alkoxyresorufin O-dealkylase (AROD) and testosterone hydroxylase (TH) activities were de-

tected in both seal species although not in some samples. Seal microsomes could metabolize ethoxyresorufin at a higher rate than other alkoxyresorufins compared to the rat microsomes. In contrast to rat, seal microsomes could hydroxylate testosterone at 2β -, and 16β -positions, although 2α -, 7α -, 16α -hydroxy testosterone and androstenedione were not produced. (3) Western blotting analysis showed the presence of Rat CYP1A1-, 1A2-, 2B (single band detected)-, and 3A (two bands detected)-like proteins in both seals. The CYP2B-like protein band in seal microsomes was apparently weaker than untreated rat CYP2B, suggesting that less CYP2B-like protein was produced or immunodetected by the rabbit anti-rat CYP2B1 antibody. (4) Correlations among AROD activi-