BIO-MEDICAL SCIENCES

Biochemistry and Biophysics
14. Enhancement of Early Retrotransposon (ETn) RNA in Myeloid Leukemia Cells from C3H/He Mice

Hiroshi Ishihara and Izumi Tanaka

Keywords: retrotransposon, gene expression, early-transposon, VL30, intracisternal A-particle, DNA-protein binding

Acute myeloid leukemia (AML) cells from C3H/He mice induced by radiation have an active retrovirus-like retrotransposon, the intracisternal A-particle (IAP) element. Examples of an accumulation of IAP RNA and genomic aberration by the IAP-mediated retrotransposition in AML cells were reported previously. Since the function of the retrotransposon is inhibited in normal and most tumor cells, the AML cells may lack mechanisms to repress the retrotransposons. To confirm this possibility, we analyzed the transcription of other mouse retrotransposons, the early retrotransposon (ETn) and virus-like 30S (VL30) particle elements in the AML cells.

All the AML lines from different mice commonly overexpressed the ETn RNA (Fig. 15 (a) ). The ETn is known as an inactive transposon since the expression is limited to only early embryonic cells. As with other tumor cells, only faint levels of ETn RNA were detected in the cells from other tumors including hepatoma and lymphoma from C3H mouse. By structural analyses of ETn cDNA clones, it was revealed that the ETn RNA molecules overexpressed in AML cells had complete forms except for a larger variation in the polyadenylation sites at the 3’-end of the molecule.

To determine the nucleotide sequence that contributes to the transcription of ETn, electrophoretic mobility-shift assay using the long-terminal repeat sequence of ETn and the nuclear extract of the AML cells was performed. The target sequence against the nuclear protein was the C-rich nucleotide sequence positioned 30 nucleotides upstream from the transcriptional start site (Fig. 15 (b) ). The C-rich sequence did not have any similarity with previously reported target motifs for enhancers.

Since ETn does not have any open-reading frame, a biological effect due to the overexpression can be ignored in AML cells. However, this finding shows that the cells of all the leukemia lines are activated by a common mechanism that simultaneously transcribes ETn. The activation of this mechanism is probably necessary for the leukemic transformation. Thus the ETn LTR function is useful to study the leukemogenetic process in myeloid cells.

Publication:

Fig. 15. Overexpression of ETn in AML cells and binding of ETn LTR sequence to nuclear extracts. (a). RNAs from normal liver cell from 2 different mice, 4 lines of primary hepatic tumor cells, 6 lines of primary AML cells, 4 lines of AML strain cells and 4 lines of primary thymic lymphoma cell were used in northern analysis probing ETn. (b). Mixtures of the end-labeled double-stranded oligonucleotides (block-1 to 11, shown in numbered boxes on both sides of the arrow) corresponding to ETn LTR (arrow) and nuclear extract from spleen (S) or leukemia lines (L1 or L2) were used for the EMSA assay until free DNAs (F) were overrun from the gel. Areas of gels where bands are retarded are shown as (B). Nucleotide sequence that can bind to the nuclear protein (corresponding to block-7 to 9) is indicated at the bottom.
15. In Vivo Radioprotection of Stable Free Radical Nitroxides, Carbamoyl- and Methoxycarbonyl-PROXYL against Whole Body X-Ray Irradiation of Mice

Kazunori Anzai, Masako Furuse, Hiroshi Ishihara and Nobuo Ikota

Keywords: radioprotection, stable nitroxide radical, X-ray irradiation, mice

Radiation induced biological damages are thought to be initiated and propagated via free radical reactions. Therefore, antioxidants are possible candidates for the radiation protection agents. Among these antioxidants, stable nitroxide radicals are interesting because they have superoxide dismutase-like activity. In addition, the radiation protection activity of stable nitroxide radicals has been reported. Methoxycarbonyl-PROXYL (MC-PROXYL) is one of the stable nitroxide radicals. It has a unique property; it is moderately lipophilic and blood-brain-barrier permeable. Previously, we have demonstrated the distribution of MC-PROXYL to mouse brain using autoradiography and in vivo ESR. In the present study, we examined in vivo radiation protection activity of MC-PROXYL against whole body X-ray irradiation of mice in comparison with carbamoyl-PROXYL, a similar but more hydrophilic stable nitroxide radical.

Mice (C3H, male, 10 weeks old) were placed in a chamber after the i.p. administration of MC-PROXYL (450 mg/kg body wt.) and were X-ray irradiated (8.0 Gy, 0.6 Gy/min) at 5 min after the administration of MC-PROXYL. MC-PROXYL increased the survival rate from 0% to 40-50%, showing radiation protection activity. The survival rate was dependent on the timing of the administration and the dose of MC-PROXYL. The condition of i.p. administration at 5 min before the X-irradiation and 450 mg/kg body wt. was the best. A higher dose of MC-PROXYL than 450 mg/kg body wt. caused acute toxicity. MC-PROXYL (450 mg/kg body wt.) increased the LD_{50/0} from 6.7 Gy to 8.0 Gy, yielding the dose reduction factor (DRF) of 1.2. This radiation protection activity of MC-PROXYL was larger than that of carbamoyl-PROXYL (DRF = 1.1). The distribution of carbamoyl- and MC-PROXYL to the bone marrow was measured by using ESR. At 5 min after the i.p. administration of carbamoyl- or MC-PROXYL, the mice were killed and bone marrow cells were collected from the thigh bones. ESR spectra of the cell suspensions after oxidation by 1 mM K,Fe(CN)_{6} showed that the distribution of MC-PROXYL in the bone marrow was similar for MC-PROXYL and carbamoyl-PROXYL. Therefore, the difference in radioprotection was not due to any difference in distribution of the chemicals to the bone marrow. A combined administration of MC-PROXYL with heat-killed Lactobacillus casei preparation (LBC) further increased the radiation protection activity. Since the concentration of LBC used in this experiment was in the range showing saturation effect, this finding suggested that the radio-protection mechanism of MC-PROXYL was different from that of LBC.

16. Hydroxyl Radical Formation Mediated by Spin Trapping Agent, DMPO, in Uroporphyrin Photodynamic Reaction in the Presence of Biological Reductants

Keizo Takeshita, Chiho Nishizawa*, Jun-ichi Ueda, Kazuo T. Suzuki* and Toshihiko Ozawa
(* Graduate School of Medical and Pharmaceutical Sciences, Chiba University)

Keywords: hydroxyl radical, singlet oxygen, spin trapping, DMPO, DEPMPO

The photodynamic reaction has been actively used for the treatment of malignant disease (photodynamic therapy, PDT). The oxidation of biomaterials caused by reactive oxygen species (ROS) is believed to be one of the potent mechanisms of PDT. The spin trapping/ESR technique with 5,5-dimethyl-1-pyrrole N-oxide (DMPO) has been commonly used to detect oxygen radicals. However, the reaction of DMPO in photosensitization has not been characterized enough. We observed that the presence of reducing agents such as NADPH, glutathione and Trolox (a water-soluble tocopherol derivative) remarkably increased the ESR signal of hydroxyl radical (·OH) adduct of DMPO (DMPO/OH) in the uroporphyrin photosensitizing reaction with visible light. The inhibition experiments with ROS scavengers indicated that the formation of DMPO/OH results from singlet oxygen ('O_{2})-mediated generation of free ·OH. When DMPO was replaced with 5-(diethoxycarbonyl)-5-methyl-1-pyrrole N-oxide (DEPMPO), neither NADPH, glutathione nor Trolox increased the ESR signal of ·OH adduct of DEPMPO (DEPMPO/OH). However, the addition of DMPO to the reaction mixture together with DEPMPO remarkably increased the signal of DEPMPO/OH regardless of the presence of the reducing agents, accompanying a distinct DMPO/OH signal only in the presence of the reducing agents. The production of ·OH was also determined with hydroxylation of salicylic acid. The presence of DMPO increased the amount of 2,3-dihydrobenzoic acid (a product of ·OH reaction with salicylic acid). The increase in the presence of the reducing agent was
almost the same as that in the absence of the reducing agent. These results indicated that the \( \cdot O_2 \)-mediated \( \cdot OH \) formation occurs DMPO-dependently to form DMPO/\( \cdot OH \) regardless of the presence of the reducing agents, and that the DMPO/\( \cdot OH \) declines in the absence of reducing agent.

17. Acute Induction of Heme Oxygenase-1 in Rat Liver by a Whole-body X-ray Irradiation

Keiko Suzuki, Masahiko Mori, Fumihiro Kugawa* and Hiroshi Ishihara
(*Nihon Univ.)

**Keywords:** heme oxygenase, X-rays, liver

The transcription of the heme oxygenase-1 (HO-1) gene is enhanced by various stimuli, such as oxidative stress, UVA radiation and heat shock, and a considerable body of evidence has confirmed that the HO-1 gene is cytoprotective against numerous stresses. The HO-1 gene is also commonly called Hsp (heat shock protein) 32. In mammals, biliverdin, which is one of the products of an enzyme reaction by heme oxygenase, is subsequently converted to bilirubin by biliverdin reductase. Both biliverdin and bilirubin are powerful antioxidants. Another product of the enzyme reaction by heme oxygenase is carbon monoxide, which is considered to be a promising and significant messenger molecule in the soluble guanylate cyclase (sGC)-cGMP system.

No immediate early effect of radiation upon the induction of HO-1 enzyme has been characterized, yet. Now, in the present study we have provided evidence that the heme oxygenase-1 enzyme is induced by ionizing radiation in rat liver within a few hours.

When male Wistar MS strain rats (8 weeks) received whole-body X-ray irradiation of 17.0 Gy, the activity of heme oxygenase in the liver was significantly enhanced (2.5 times) 7 h later (Fig.16). Western blotting of the irradiated liver demonstrated a significant increase in the level of HO-1 protein. The level increased 2 h after the irradiation, reached a peak at 4 h, and then decreased gradually until 10 h, when it was still higher than the control level. Thus, X-rays were confirmed to be stressors that induce acute HO-1 expression transiently in the liver.

18. Involvement of Protein Kinase C in Radiation-Induced Apoptosis Signaling Pathways in Murine Thymic Lymphoma Cells (3SBH5 Cells)

Tetsuo Nakajima, Osami Yukawa, Chihiro Azuma*, Harumi Ohyama, Bing Wang, Shuji Kojima*, Isamu Hayata and Hiroko Hama-Inaba
(*Science University of Tokyo)

**Keywords:** murine thymic lymphoma, protein kinase C, apoptosis, radiosensitivity, Raf-1

Radiation-induced apoptosis is known to be important for understanding the mechanism of radiosensitivity. It is widely accepted that protein kinase C (PKC) participates in the regulation of radiation-induced apoptosis. However, the PKC function remains obscure in the mechanism of radiosensitivity. Using 3SBH5 cells, one of the radiation sensitive murine thymic lymphoma cells were used to assess involvement of PKC in radiation-induced apoptosis regulation. 3SBH5 cells are quite sensitive to X-rays and undergo apoptosis shortly after X-ray-irradiation. PMA (phorbol 12-myristate 13-acetate), an activator of PKC, blocked the radiation-induced apoptosis in 3SBH5 cells. In contrast, chelerythrine, a PKC inhibitor, enhanced the radiation-induced apoptosis, as did Gö6976, a classical PKC (cPKC) specific inhibitor, for irradiation with 0.5Gy. These results imply that cPKC contributes to the balance between cell survival and death through radiation-induced activation of cPKC. Furthermore, irradiation alone had no effect on the distribution of PKC subtypes in 3SBH5 cells. This suggests that the radiation-induced cPKC activation is not caused by translocation of PKC molecules. On the other hand, although it was demonstrated that cPKC is associated with an anti-Raf-1 antibody-recognized protein in 3SBH5 cells, Raf1 Kinase Inhibitor I, one of the Raf1 kinase inhibitors, had no effect on the radiation-induced apoptosis. The anti-apoptotic function of cPKC in the radiation-induced
apoptosis is under investigation by exploring cPKC-associated proteins. This function of cPKC may offer a clue to understanding radiosensitivity and radiation-induced adaptive response.

19. Difference among Heavy Ion Beams in Cell Killing and Mutation Induction

Yoshiya Furusawa, Tatsuki Kanai, Naoyuki Shigematsu¹, Noriko Ihara², Tetsuya Kawata³, Osamu Kawaguchi³, Atsuya Takeda¹, Ryoichi Ishibashi¹, Shoji Kutsuki¹, Atsushi Kubo¹, Koichi Isobe⁷, Takashi Uno⁷ and Hisao Ito⁷
(‘Keio Univ.; ’Chiba Univ.)

Keywords: cell killing, mutations, heavy ion beam

In this study, human cancer cell lines were used to attempt to clarify the radiobiological effects of heavy ion beams on carbon beam radiotherapy. Killing efficiency was determined by the usual colony forming method, and mutation was observed as the induction of 6-thioguanine resistant colony. Cells were irradiated by HIMAC carbon ion beams with LET value at 20 or 80 keV/μm, or neon ion beams at 80 keV/μm. Carbon beams of 80 keV/μm had an enhanced cytotoxic effect compared with those of 20 keV/μm. The carbon beams of 80 keV/μm showed almost the same efficiency in cell killing compared with neon beams even though they had the same LET value. Efficiencies of induction of mutation for all heavy ion beams tested were significantly higher than that for X-rays for all cell lines used. The efficiency for 80 keV/μm carbon beams was higher than that for 20 keV/μm beams. The efficiency was, however, lower for neon ion beams than carbon beams at 80 keV/μm, even though they had the same LET value. Split dose irradiation of carbon beams showed no effect on cell killing but the mutation efficiency was lowered. Neon beams might be more appropriate for cancer therapy with heavy ion beams, and the fractionated dose might be appropriate to reduce the mutation frequency in heavy ion radiotherapy.

Publication:

20. Medium-Mediated Bystander Effects on HSG Cells Co-Cultivated with Carbon Beam-Irradiated Cells

Yoshiya Furusawa, Chunlin Shao and Mizuho Aoki

Keywords: bystander effect, heavy-ion beam, micronucleus, nitric oxide

The mechanisms of medium-mediated bystander effects on cell survival and micronucleus (MN) induction were investigated by co-cultivating unirradiated HSG cells with cells irradiated by X-rays or 290 MeV/u carbon beams. It was found that the survival of the irradiated cells exponentially decreased along with the dose, and that the plating efficiency (PE) of the unirradiated recipient cells was clearly more enhanced than that of the control cells. Moreover, MN was induced in the unirradiated recipient cells and its yield had a maximum distribution corresponding to the donor dose, which was different from the linear-quadratic dose response of the yield of MN in the irradiated cells. The treatment of PTIO, a scavenger of nitric oxide (NO), decreased both PE and MN of the unirradiated recipient cells to control levels. Moreover, nitrite was detected in the co-culture medium, and its concentration was related to the donor dose. These results indicated that NO was involved in the above mentioned medium-mediated bystander effects. In addition, an equation was deduced which well fit the induction of MN of the unirradiated recipient cells.

Publication: