A－4．2．2 Effect of ultraviolet ray increase on human health Effects on promotion of cancer

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ABSTRACT

Our objectives are to investigate the expression of newly induced protein in UVB exposed mice or cultured cells, to clarify modulation of mast cell differentiation from UVB exposed bone marrow cells and to extablish a new mutation assay system. The results obtained are as follows: 1) The methods to detect the expression of a variety of new synthesized proteins in UVB irradiated cells were determined. 2) Although the growth of mast cells was suppressed by UVB irradiation, the expression of IgE receptor on mast cells was increased when compared to those from control. 3) Retroviral vector LTK-15 constructed with HSV-TK and neo genes was established for detecting the mutation caused by UVB. 4) We have established a skin cancer model by using hairless mice. The model could be utilized to study mechanisms of gene expression by UVB irradiation and those of stress-mediated protein synthesis. 5) It was shown that the effects of active oxygen species, which are produced by UV irradiation in a single-exposure study, can be suppressed if MT or GSH levels can be increased in the tissues. On the other hand, in a repeated-exposure study, a decrease in endogenous GSH and an increase in MDA do not occur, suggesting the presence of an adaptation of skin tissues to UVB irradiation.

Key Words Skin Cancer, Metallothionein, Mutation, Gene, Mast Cell

1) Detection of gene products expressed during cell proliferation.

The gene products induced during cell proliferation were expected to be expressed in the cells exposed to UVB light as well as tumor promoters.

① We determined the methods to detect the expression of c-jun, proto-oncogene, metallothionein (MT), which concerned to metabolism of heavy metals during cell proliferation, and heat shock proteins (HSP-90, HSP-70 and HSP-27) induced by various environmental stresses.

Methods:

Northern blotting method was used to analyse the m-RNAs. Western blotting method and immunoprecipitation were applied to determine the amount of each protein.

Results:

c-jun m-RNA can be analyzed with enough sensitivity to detect its

induction by cell growth factors, and amount of c-jun polypeptide was estimated by immunoprecipitation with protein A. Isoproteins of MT can be detected by western blotting with detection limit of 0.06 ug. Amounts of HSP-90, HSP-70 and HSP-27 m-RNAs and HSP-70 polypeptide were determined by northern blotting and western blotting, respectively.

② We examined whether HSPs were expressed by UVB irradiation.

In vitro study.

Methods:
After HeLa S3 cells were exposed to UVB light, newly-synthesized proteins were labelled with [^{35}S]methionine. Cellular proteins were solubilized by a detergent solution, and were separated by two dimensional gel electrophoresis. Separated proteins were detected by autoradiography.

Results:
A protein with molecular weight of 70,000 (70kD protein) was induced 12 hrs after irradiation of UVB at 300 J/m^2. This protein was also induced by heat treatment at 45℃. The proteins separated on the gel were transferred to a PVDF membrane, and 70kD protein was identified as HSP-70 by immunochemical staining. Synthesis of several proteins, other than HSP-70, were stimulated by UVB, but these proteins remains to be determined.

In vivo study.

Methods:
After hairless mice were once exposed to UVB light at 15k J/m^2, their epidermis were solubilized using polytron homogenizer. Solubilized proteins were separated on two dimensional gel, and separated proteins were stained by a dye.

Results:
Amount of HSP-70 was increased by UVB irradiation, suggesting to be a good marker for the irradiation. However, since its induction should result from acute exposure of UV-B, further study is required to determine the marker for chronic exposure, which is important as an indicator of human health.

2) Development of new assay system for detecting the mutation using Herpes simplex virus thimidine kinase (HSV-TK) gene.

A retroviral vector carrying both positive (neo) and negative (HSV-TK gene) selection markers was transfected to rat fibroblast cells. Since HSV-TK, but not the host TK, is capable of converting ACV to a toxic metabolite, cells rataining intact HSV-TK gene fail to survive, while the cells carrying mutated HSV-TK gene or which have lost the gene can form colonies in the presence of ACV, making it possible to detect the genetic defects in a positive manner. By UVB irradiation, the colony formation was stimulated dependent on UVB

dose, and at a dose of 36 J/m^2 the number of colony was 15.6 times higher than non-irradiated cells. This system will be applicable for a wide variety of mammalian cells and provide a useful tool to assess their susceptibility to various mutagens and their genomic instability.

3) Effects of 300nm UVB on mast cell growth and differentiation.

The evidence of mast cell involvement in immunoregulation, inflammation as well as immediate hypersensitivity has been accumulated. To clarify the modulation of cell growth and differentiation by UVB irradiation, effects of UVB iradiation on mast cell growth and differentiation were investigated.

Methods:

Mouse bone marrow cells (BMC) or differentiated mast cells were irradiated with various doses of 300nm UVB. To differentiate mast cell population, adherent cells to culture plate were discarded and nonadherent cells were transferred into fresh medium containing 25% WEHI-3 conditioned medium every weeks. On day 7, 14 and 21, the number of alcian blue positive mast cells, histamine content and expression of IgE receptor were measured.

Result and Discussion:

UVB irradiation on BMC did not show any changes in the number of total nonadherent cells after the irradiation. The number of alcian blue positive cells was significantly decreased by 140 and 700J/m2 UVB irradiation. Total histamine content was not increased in BMMC differentiated from UVB irradiated BMC. However, UVB irradiation significantly increased expression of IgE receptors on membrane of differentiated BMMC. On the other hand, UVB irradiation on mast cells significantly reduced the number of cells and increased the expression of IgE receptors. These results showed that 300nm UVB irradiation affected the growth and differentiation of mast cells.

4) Development of skin cancer model in hairless mice by UVB iradiation

Introduction:

It has been considered that ultraviolet B (UVB) and A acts as an initiator and a promotor for the development of skin cancer, but the details remain to be fully studied. In this particular study, we have tried to develop UVB-irradiated skin cancer model by utilizing hairless mice so that the animals can be used for various studies in the present research project.

Methods:

Hairless mice were exposed to 2 kJ/m2/day, 3 times a week for 25 weeks. Histological observations and several biochemical analyses were performed.

Results:

Repeated exposure to UVB irradiation caused erythema, dryness and thickening in the skin in the second week. Solar keratosis was observed in the 8th week, which developed to squamous carcinoma in

some mice in the 25th week.

Discussion:

We have confirmed the skin carcinogenesis in hairless mice after prolonged exposure to UVB. This model could be utilized to study mechanisms of gene expression by UVB irradiation and those of stress-mediated protein synthesis.

5) Reduction of the damage caused by active oxygen species.

Exposure to ultraviolet ray irradiation results in the formation active oxygen species like hydroxyradicals in the cell. In this study, we have investigated mechanisms how endogenous SH compounds in the living cells modify possible deleterious effects of the oxidative stress.

Materials and methods:

Hairless mice were exposed to UVB once (6 and 12 kJ/m2) or 3 times/week for 25 weeks (2 kJ/m2/day). Each series of experiments consist of several groups of animals which were treated as follows: (a) no treatment, (b) UVB, (c) Zn + UVB, (d) GSH ester, (e) GSH ester + UVB, (f) GSH and (g) GSH + UVB. In the single exposure and repeated exposure studies, mice were sacrificed 24 hours after the UVB irradiation. Skin were collected and biochemical analyses were performed. As an in vitro study, we have irradiated UVB to NB1RGB and HeLa S3 cells and studied a possible protective effect of MT found in vivo.

Results:

In the single exposure study, a decrease in GSH and an increase in MDA were observed in response to UVB irradiation. This was suppressed by GSH ester and Zn treated groups. In the group (g), MT was found to be increased. These results suggest that SH residues in GSH and MT play a role in reducing the effects of active oxygens. On the other hand, in the repeated exposure study, no increase in MDA was observed. In the cell culture study, it was found that the cells became more tolerant to UVB irradiation when they were pretreated with Zn or dexamethasone that can induce MT.

Discussion:

The effects of active oxygen species, which are produced by UV irradiation in a single-exposure study, can be suppressed if MT or GSH levels can be increased in the tissues. On the other hand, in a repeated-exposure study, a decrease in endogenous GSH and an increase in MDA do not occur, suggesting the presence of an adaptation of skin tissues to UVB irradiation.