

- 77 UVB/UVC-responsive Intracellular Signaling Pathways: A Chronological and Comparative Study.  
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In order to understand molecular mechanisms involved in wavelength-dependent cellular responses to UV radiation, we examined chronological changes of signaling molecules in UVB (312nm) or UVC (254nm)-irradiated normal human fibroblastic cells (HE49). Both UVB and UVC at a dose which caused the equivalent chronogenic death of HE49 cells formed an equal amount of DNA photoproducts. However, DNA damage-dependent p53 pathway responded earlier in UVB-irradiated cells than in UVC-irradiated cells. Furthermore, the major phosphorylation site of p53 after irradiation was ser-392 in UVB-irradiated cells whereas ser-15 in UVC-irradiated cells. Among MAPKs as DNA damage-independent pathways, UVB caused sustained phosphorylation of p38. In contrast, transient phosphorylation of JNKs was observed after UVC irradiation. These results suggested that post-translational regulation of p53, phosphorylation of p38 and JNKs, and possibly the interaction of them, differ depending on UV wavelength, although the quantitative relationship between the amount of DNA damage and the extent of cell death do not differ.

- 78 Wavelength Dependency of Apoptosis Induction and Reproductive Death upon Mammalian Cells by Monochromatic Ultraviolet Light  
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To determine biological effectiveness of the sunlight upon mammalian cells, the wavelength dependence on the induction of apoptosis and reproductive death exposed to monochromatic ultraviolet light was investigated using the Okazaki Large Spectrograph at the National Institute of Basic Biology, Okazaki. L5178Y cells were exposed to the light at different wavelengths in UV-A and -B regions, and frequencies of apoptosis and reproductive death were determined. Exposed dose to induce apoptosis in 10% cells were 24 J/m<sup>2</sup>, 180 J/m<sup>2</sup>, and 140 kJ/m<sup>2</sup> at 280, 300, and 320 nm, respectively. Action spectra of apoptosis induction and reproductive cell death were analogous to the absorption spectrum of DNA in UV-B region, but not in UV-A region. Different time-course of induction of apoptosis and different shapes of the survival curves were found between UV-B (a long time and a small shoulder) and UV-A (a short time and a large shoulder) region.

- 79 Identification of DNA-crosslinked proteins by UVB-photoactivated gilvocarcin V  
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Gilvocarcin V (GV) is an antitumor antibiotic with four-rings aromatic structure that promotes protein-DNA crosslinking when photoactivated by near-UV light (UVB). We identified proteins that are selectively crosslinked to DNA by photoactivated GV in human fibroblasts. Using the potassium-SDS precipitation method, DNA-crosslinked proteins were prepared in quantity. Using N-terminal amino acid sequencing and western blot analysis, the selectively crosslinked proteins were identified as histone H3 (13 kDa) and GRP78, a stress 70 heat-shock protein, lacking its hydrophobic leader sequence (76 kDa). Identity of these two proteins were also ascertained by a modified agarose-western blot analysis, in which the two retarded band signals originally found in native agarose gel separation of GV-treated samples<sup>a</sup>. It is of interest to note that photoactivated GV crosslinks phosphorylated form of histone H3, but does not other histones. Since the N-terminal region of histone H3 provide sites not only for phosphorylation but for acetylation, methylation and ADP-ribosylation, which are regarded as histone codes to regulate replication, cell division and gene expression, its relevance to the potent antitumor activity of photoactivated GV would be speculated.

<sup>a</sup> Matsumoto, A. et al. Photochem. Photobiol., 60, 225-230, 1994

<sup>b</sup> Matsumoto, A. & Hanawalt P.C. Cancer Res., 60, 3921-3926, 2000