

Determination of UV-induced DNA damage and photoprotection by melanin

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Melanin is believed to protect cells against ultraviolet (UV)-induced damage formation. It, however, has not been experimentally proved, because published results have been inconclusive. The present study was carried out to determine whether intracellular melanin protected cells against UV-induced photoproduct type DNA damage formation and killing. Three human melanoma cell lines containing different amounts of melanin were used. Absorption spectrum, subcellular localization of melanin, and melanin concentration were examined in the three cell lines. Two types of DNA damage, cyclobutane pyrimidine dimers and (6-4) photoproducts, were detected by an enzyme-linked immunosorbent assay (ELISA) with monoclonal antibodies specific for these photolesions. We found that melanin reduced the induction rates of both types of DNA damage in pigmented cells irradiated with UVC in a melanin concentration-dependent manner. Almost no differences in repair capacity for the two types of photolesions were observed among the three cell lines. We also found that the more highly melanotic cell lines were more resistant to UVC than the less melanotic cell lines. These results suggest that intracellular melanin plays an important role in preventing UV-induced cell killing by reducing the formation of two types of DNA damage. The three cell lines, however, might have different induction rates of UV-damage and different UV sensitivities, under identical conditions with equal amounts of melanin, since they stemmed from melanomas of three different patients. Besides, we have not ascertained whether melanin protects cells against sunlight, since we used UVC only. Thus, we treated highly melanotic HM3KO cells with docosahexaenoic acid (DHA) to reduce the amount of melanin to about half, and prepared two types of cells with the same genetic background, with different melanin levels. We carried out similar experiments using UVC and UVB, and ascertained that melanin protected melanoma cells from the two types of DNA damage formation and killing both after UVC and after UVB irradiation. Data obtained from the two studies in series suggest that the protective effect of intracellular melanin increases in a melanin concentration-dependent manner and reaches a plateau.