Role of á-MSH in Repair of UV-Induced DNA Damage, as Determined by H2AX Phosphorylation

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Melanoma is the deadliest form of skin cancer and is on the rise. An important paracrine factor that offers photoprotection against the carcinogenic effect of ultraviolet radiation (UV) is á-melanocortin (á-MSH). It not only stimulates eumelanin synthesis, a photoprotective pigment, but also reduces the generation of damaging reactive oxygen species, and enhances nucleotide excision repair (NER). á-MSH binds to the melanocortin 1 receptor, a G protein-coupled receptor expressed on melanocytes, causing an increase in cAMP. There are many different *MC1R* alleles that are expressed in different human populations. Some are non-functional variants that render melanocytes unresponsive to á-MSH. We explored the role of á -MSH on H2AX phosphorylation (ãH2AX) in melanocytes with different MC1R genotypes. H2AX is a histone protein that when phosphorylated, recruits repair enzymes to the site of damaged DNA. H2AX phosphorylation has been studied in regards to double stranded DNA breaks from ionizing radiation, but less is known about its role in repair of UVR damage. It is thought that while repairing cyclobutane pyrimidine dimers caused by UV, double stranded breaks occur, and this causes ãH2AX. Thus, we used ãH2AX as a measure of repair of UV-induced damage. To investigate the effects of á-MSH on H2AX, Western blots were run using proteins extracted from melanocytes at four time points post irradiation. Four experimental groups were used: control, á-MSH, UV, and UV+ á-MSH, and ãH2AX was detected. Flow cytometry and immunocytochemistry were also used to compare the extent of ãH2AX. The results of these experiments showed an increase in phosphorylation with UV exposure, which was augmented in the presence of á –MSH in melanocytes with functional MC1R. á-MSH had no significant effect on ãH2AX in cells with non-functional MC1R, indicating that the effect of á –MSH required functional MC1R. This preliminary study showing that á-MSH increases ãH2AX further confirms that á-MSH augments the repair of UV-induced DNA damage, a mechanism necessary to prevent mutations and the malignant transformation of melanocytes to melanoma

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