Regulation of Upstream Stimulatory Factor-1 (USF-1) in Human Melanocytes by ultraviolet radiation and α-melanocyte stimulating hormone

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Introduction: Tanning ability is a major risk factor underlying UV-induced skin cancers, including melanoma. Synthesis of melanin, both constitutively and in response to solar ultraviolet radiation (UV), is largely mediated by the melanocortin 1 receptor (MC1R), a membrane bound G_s protein- coupled receptor expressed by pigment cells, melanocytes. Upstream Stimulatory Factor-1 (USF-1), a basic helix-loop-helix transcription factor, contributes to pigmentation of mouse melanocytes and melanomas in both mice and humans via regulating the expression of genes for MC1R and proopiomelanocortin, the precursor for melanocortins, the agonists of MC1R, as well as for tyrosinase, the rate limiting enzyme in the melanogenic pathway. Whether USF-1 plays a similar role in normal human melanocytes (HM) has not yet been explored.

Aims/Hypotheses: We hypothesized that UV irradiation of HM results in phosphorylation and activation of USF-1 and that this effect is enhanced by α-melanocyte stimulating hormone (α-MSH), the agonist of MC1R.

Methods: Primary cultures of HM, each derived from a single donor, were irradiated with UVB and/or treated with αMSH. Cell lysates were extracted at various times following irradiation and used for Western blot analysis, using USF-1-specific antibody, and an antibody for actin for loading control.
To confirm the role of USF-1 in the UV response, we stably silenced USF-1 gene in HM using shRNA or infected HM with scrambled sequence shRNA, followed by selection with puromycin. These cells will be used to determine the role of USF-1 in regulating expression of downstream targets that affect the response to UV and/or α-MSH.

Results: Normal HM express three forms of USF-1 with molecular weights around 43 kDa, likely representing the unmodified, and a post-translationally modified form of the protein, and a lower molecular form, possibly representing a splice variant of the protein. The specificity of the antibody was confirmed using a blocking peptide. Irradiation with UV increased the levels of these forms in a time-dependent manner, and α-MSH contributed to this effect.

Conclusion: These data suggest that USF-1 plays a role in HM response to UV and α-MSH. Further studies need to be done to further define the role and regulation of USF-1 in HM.