

#### SNAM1-4

### **Closing the vicious cycle of skin cell photo-oxidative stress: The lipid peroxidation-derived protein epitope DHP is a potent UVA-sensitizer**

Georg T. Wondrak, Christopher M. Cabello, Bobbi L. Anglin, Sara M. Azimian, Sarah D. Lamore

*University of Arizona, College of Pharmacy and Arizona Cancer Center, Tucson, AZ, United States*

Light-driven electron and energy transfer involving non-DNA skin chromophores as endogenous photosensitizers induces oxidative stress in UVA-exposed human skin, relevant to photoaging and photocarcinogenesis. Here we present experimental evidence that the malondialdehyde (MDA)-derived protein epitope dihydropyridine (DHP) is a potent endogenous UVA-photosensitizer of human skin cells. MDA is an important electrophilic carbonyl species derived from membrane lipid peroxidation. MDA-derived protein epitopes are found in human tissue under oxidative stress and accumulate in melanoma and non-melanoma skin cancer. Using the protected dihydropyridine-derivative (2S)-Boc-2-amino-6-(3,5-diformyl-4-methyl-4H-pyridin-1yl)-hexanoic acid t-butylester as a model of peptide-bound DHP, photodynamic inhibition of proliferation and induction of apoptosis were observed in cultured human skin fibroblasts and keratinocytes exposed to the combined action of low doses UVA (5 J/cm<sup>2</sup>) and DHP (10 μM). DHP-photosensitization induced intracellular oxidative stress, dramatic upregulation of hemeoxygenase-1 expression, and formation of TBARs from lipid peroxidation. Consistent with UVA-driven ROS generation by DHP, superoxide formation was detected, but little protection was achieved using SOD or catalase during cellular photosensitization. In contrast, inclusion of NaN<sub>3</sub> completely abolished DHP-photosensitization. Singlet oxygen involvement in DHP-mediated photooxidation was further examined by MALDI-MS-MS analysis of the model peptide melittin. DHP-UVA treatment of melittin generated a single peptide-photoproduct characterized by a 32 Da mass shift assigned to peroxidation of Trp-19 by singlet oxygen attack. Our data support the hypothesis that MDA-epitopes are key components of a vicious cycle of skin photooxidative damage involving initial lipid peroxidation, subsequent accumulation of MDA-protein epitopes, and amplification of photo-oxidative stress by DHP-photosensitization. Supported in part by NIH (SWEHSC pilot research grant [ES06694]) and ABRC (0721).