In vitro delivery of CPD-specific photolyase-encoding mRNA prevents UVB-induced mitochondrial changes

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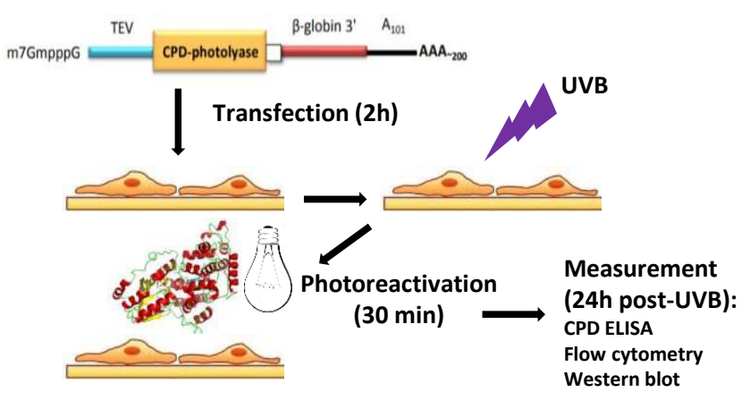
INTRODUCTION

Exposure of skin to ultraviolet radiation B (UVB) causes direct DNA damage via cyclobutane pyrimidine dimer (CPD) formation. These photolesions lead to diverse cellular and clinical effects, including inflammation, sunburn cell formation, photoaging and skin cancer, but their involvement in the regulation of mitochondrial function is unexplored. In mammals, elimination of CPDs is accomplished by the slowly-progressing nucleotide excision repair (NER). In contrast, active photolyase can rapidly remove CPDs from DNA, but have disappeared from placental mammals.

METHODS

Here, we characterized the impact of UVB-induced DNA damage on mitochondria, using our established model system, in which HaCaT keratinocytes are transfected with 1-methylpseudouridine-modified mRNA encoding CPD-photolyase. Keratinocytes were irradiated with UVB and immediately subjected to photoreactivating light required for the activation of the enzyme, or kept in dark, where photolyase is inactive.



RESULTS

Figure 1. Photoactivated photolyase removes CPDs and prevents G_2/M cell cycle block after UVB

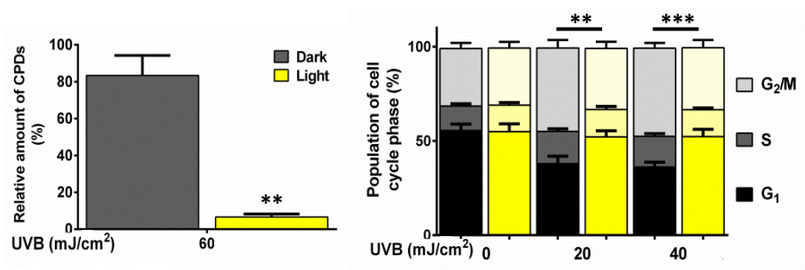


Figure 2. Photolyase activation reduces UVB-induced PARP activation

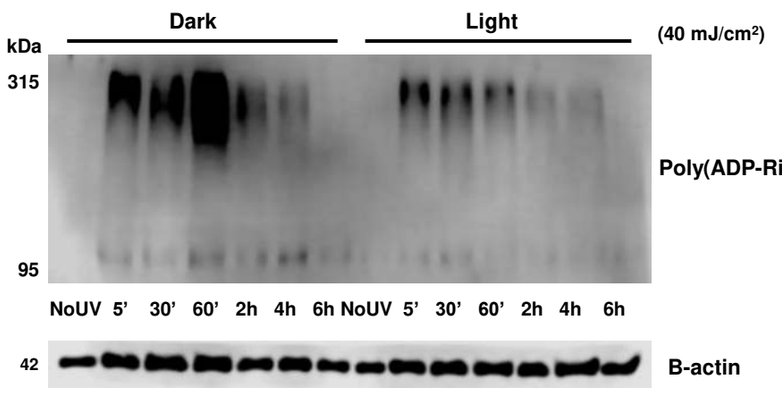


Figure 3. CPD removal diminishes UVB-caused bulk autophagy

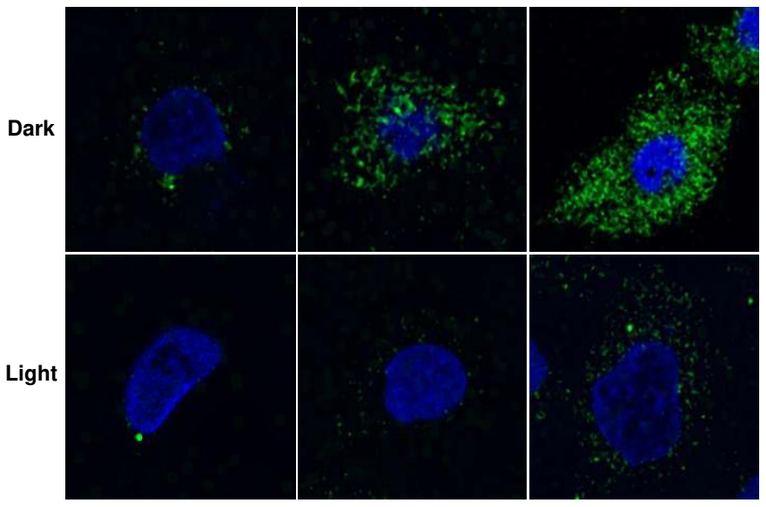


Figure 4. Photoreactivated photolyase restores cell viability and cell proliferation after UVB

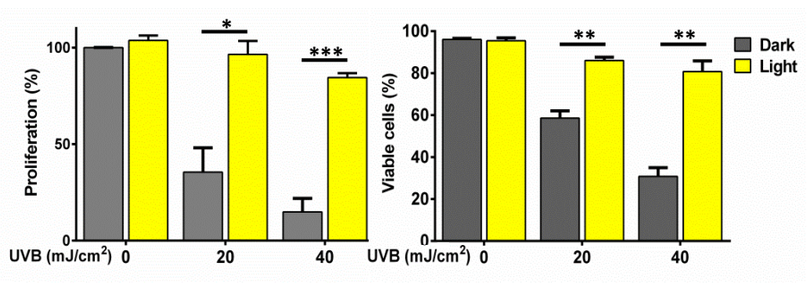
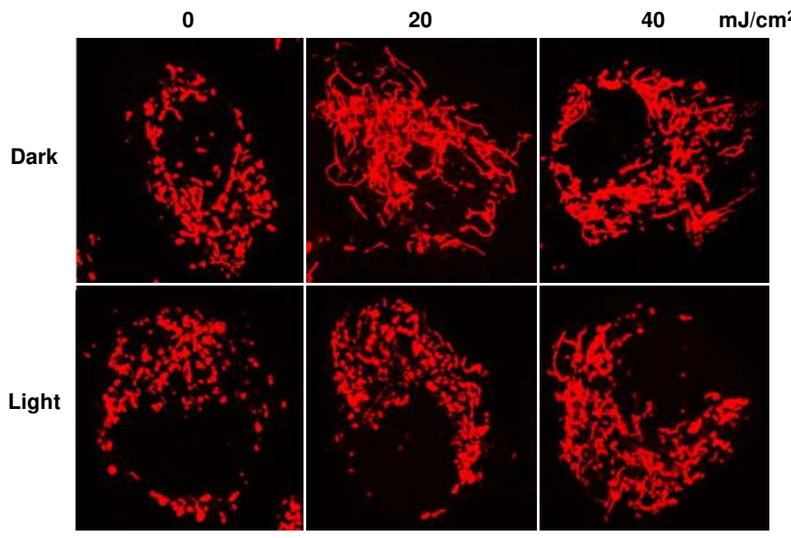


Figure 5. Photoreactivated photolyase counteracts UVB-induced mitochondrial fusion



RED: mitochondria, Mitotracker Red CMXROS

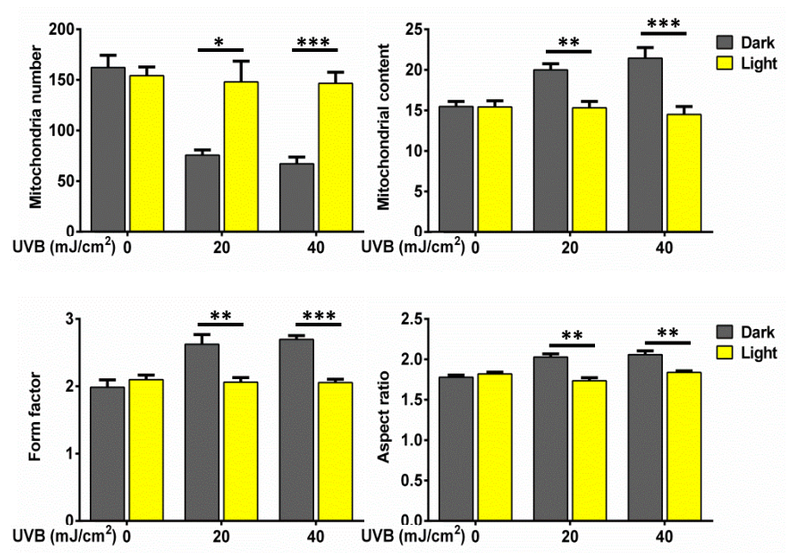


Figure 6. Photolyase activation prevents elevation in mitochondrial mass and mitochondrial biogenesis

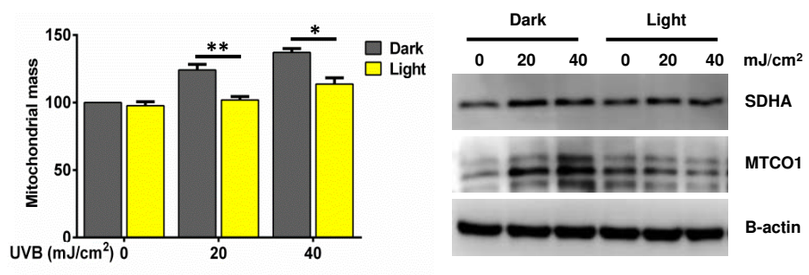


Figure 7. CPD removal reduces mitochondrial membrane hyperpolarization and ROS production

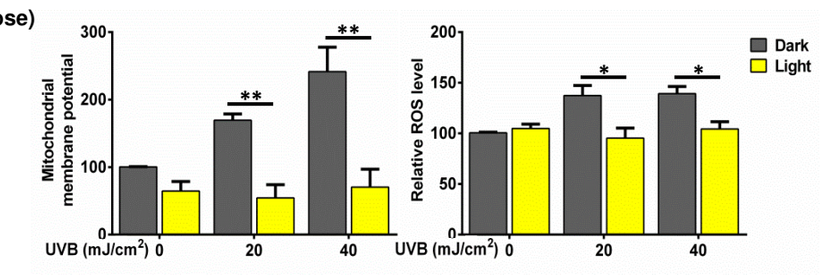
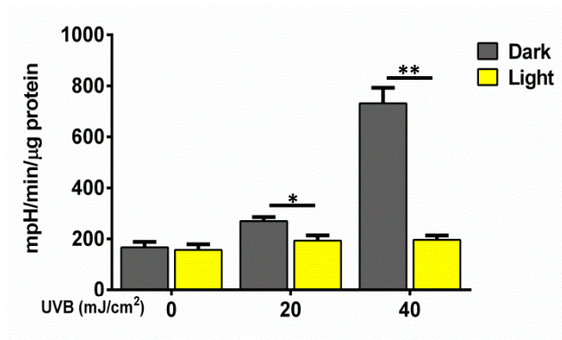


Figure 8. Photoactivated photolyase diminishes glycolysis and oxidative phosphorylation



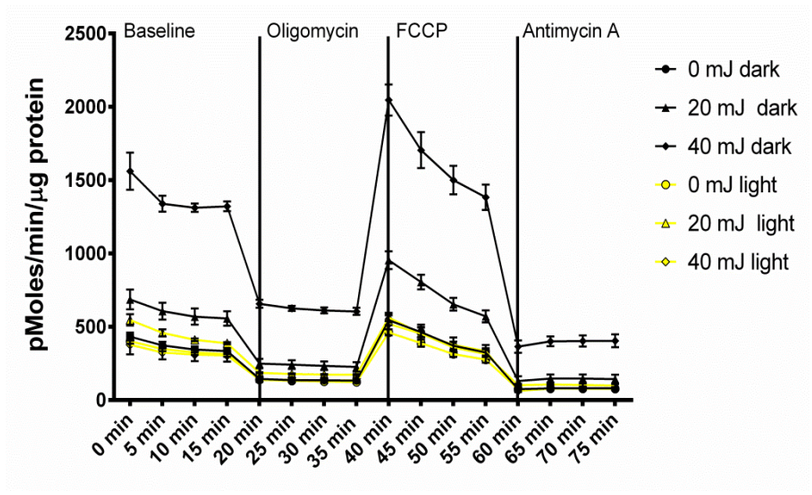


Figure 9. Oxidative phosphorylation is mediated by ATM, AMPK, p53, AKT and mTOR kinases after UVB

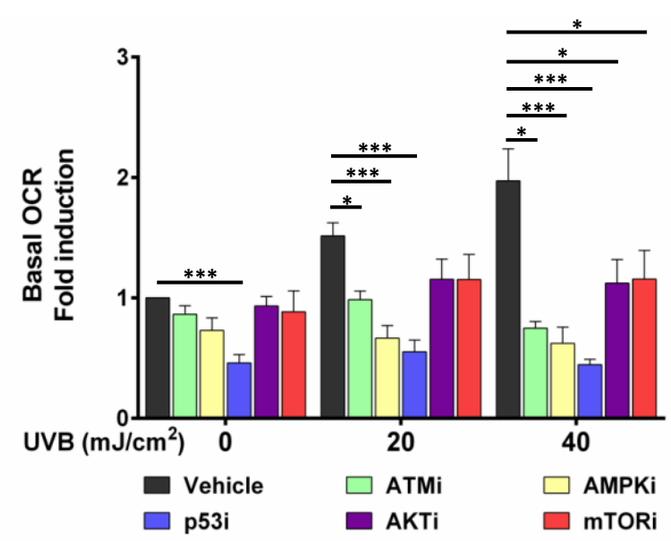


Figure 10. CPD removal decreases the phosphorylation status of proteins involved in the regulation of OXPHOS

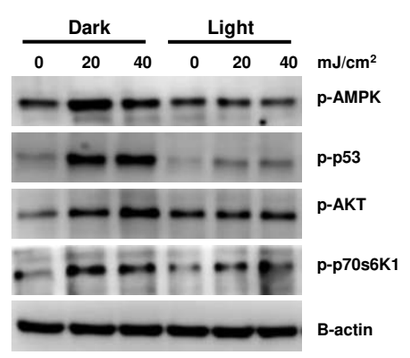


Figure 11. Photolyase activation prevents the utilization of endogen and exogen fatty acids

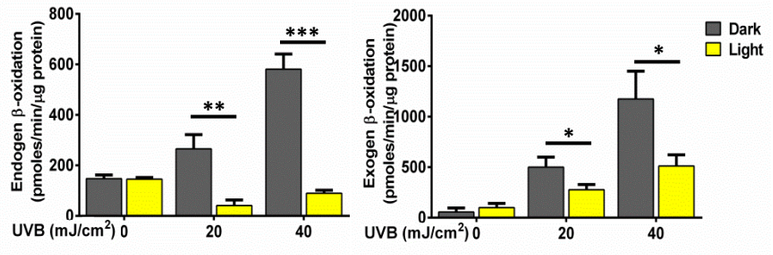
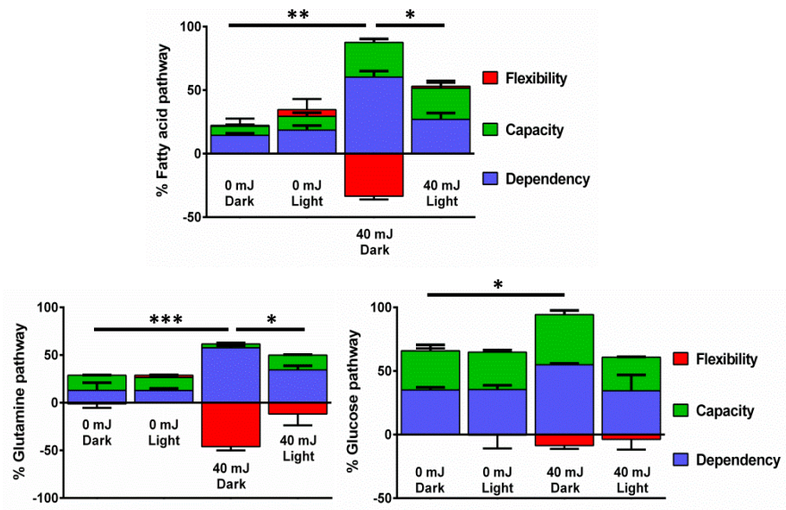


Figure 12. Metabolic burden on fatty acid and glutamine metabolism is mitigated by CPD removal



CONCLUSIONS

Our results demonstrate that CPD removal by photoactivated CPD-photolyase in HaCaT cells reduces PARP activation and bulk autophagy. Moreover, the CPD-photolyase prevents G_2/M cell cycle arrest, restores cell proliferation and cell viability. Removal of CPDs also prevented the UVB-mediated increase in mitochondrial mass, mitochondrial biogenesis and morphological changes of mitochondria. As a result of photolyase activity, mitochondrial membrane potential, superoxide production, glycolysis, oxidative phosphorylation and endogen fatty acid oxidation were all reduced to baseline level. We also demonstrated that following removal of CPDs the expression of the upstream regulators of oxidative phosphorylation and metabolic burden on fatty acid and glutamine metabolism was decreased thus suggesting that **UVB-initated DNA damage induces nucleus-to-mitochondria signaling.**

In conclusion, delivery of CPD-photolyase mRNA into cultured human keratinocytes provides a protective effect not only against DNA damage but also prevents morphological and functional changes of mitochondria after UVB exposure.

ACKNOWLEDGEMENTS

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