Synopsis of Original Research Paper

Three-dimensional visualization of ultraviolet-induced DNA damage and its repair in human cell nuclei

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Two major DNA damage produced by 254-nm UV light are cyclobutane pyrimidine dimer (CPD) and (6-4) photoproduct (6-4PP). Both photolesions are repaired in normal human cells by nucleotide excision repair (NER). However, little is known about where CPDs are repaired or where 6-4PPs are repaired in relation to the various sub-nuclear structures. The present study was performed to aim at three-dimensional demonstration of UV-induced DNA damage and its repair in human cell nuclei. We first investigated the repair kinetics of CPD and 6-4PP using an enzyme-linked immunosorbent assay (ELISA) with damage-specific monoclonal antibodies in normal human and xeroderma pigmentosum complementation group C (XP-C) cells. We also examined the kinetics of repair DNA synthesis (unscheduled DNA synthesis, UDS) using a quantitative immunofluorescence method with anti-5-bromo-2'-deoxyuridine antibodies. We confirmed the normal repair in normal human cells and the impaired repair in XP-C cells. Then, using laser scanning confocal microscopy, we succeeded in three-dimensional visualization of the nuclear localization of CPDs, 6-4PPs and UDS in individual human cells. The typical three-dimensional images of photolesions or UDS at various repair times reflected well the repair kinetics obtained by ELISA or immunofluorescence. The important finding is that the punctate, not diffusely spread, nuclear localization of unrepaired 6-4PPs was found at 2 h after irradiation. Similarly, the focal nuclear localization of UDS was observed both during the first and the second 3h-repair periods. The present results suggest that both 6-4PPs and CPDs are non-randomly repaired from nuclei in normal human cells.