Synopsis of Original Research Paper

Elucidation of Mutagenesis Mechanism by UVA Using a Novel Mutagenesis Assay

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UVA increases the risk of human cancer. One of the mutation patterns, mainly found in cancers derived from tissues directly exposed to UVA, is attributable to misreplication of DNA damage caused by 7,8-dihydro-8-oxyguanine(8-oxoG). However, little is known the mechanism of UVA mutagenesis. I previously developed the piggyBlock system. The system employs the random integration of UV damage (CPD) into the genome of cells using the 'piggyBac' transposon-based vector assay. I measured the mutation rate in human TK6 cells at a chemically synthesized 8-oxoG and CPD integrated into genomic DNA. I transfected the piggyBlock vector carrying CPD into *wild-type*, *RAD18*$^{-/-}$, and *POLη*$^{-/-}$ cells. I then selected cells with puromycin, PCR amplified nucleotide sequences over the CPD site in individual puror clones. I found that the mutation rate of *RAD18*$^{-/-}$ cells was comparable to that of wild-type. Phenotype of RAD18-deficient cells is almost all normal, in terms of the bypass of CPD site, suggesting that some redundant pathways might operate the mutation induced by CPD.

Topoisomerase I (TOP1) resolves DNA topology during replication and transcription. The enzyme forms an intermediate TOP1 cleavage complex (TOP1cc) through transient TOP1–DNA-protein crosslinks. Some anticancer drugs and carcinogen freeze this reaction intermediate. However, it is not known whether UVA generates TOP1cc. Thus, I developed an assay system to estimate the TOP1cc using anti-TOP1 antibody. In future, I will analyze the TOP1cc removal mechanism using this assay system genome editing cells.