1. No.
   2020Y002

2. Title
   Elucidation of Mutagenesis Mechanism by Tabaco Smoking Using a Novel Mutagenesis Assay and Human Genome Editing Cells

3. Period
   2020-2022

4. Summary

   Tobacco smoking increases the risk of human cancer. One of the mutation patterns, mainly found in cancers derived from tissues directly exposed to tobacco smoking, is attributable to misreplication of DNA damage caused by tobacco carcinogen. However, little is known the mechanism of tobacco mutagenesis. We clarified that the mutation rate of *POLη*$^{-/-}$ cells was comparable to that of *RAD18*$^{-/-}$ *POLη*$^{-/-}$ cells in terms of UV DNA damage. Furthermore, I also focused on the TOP1-DNA crosslinks repair. It is known Topoisomerase1 (TOP1) is trapped on DNA at the 3' end after treatment with Benzo[a]pyrene (BP). However, the repair mechanism is still unknown. To this end, I lysed human cells and separated TOP1cc from free TOP1 by sedimenting cell extracts through a CsCl gradient. Two cycles of sedimentation were used to ensure complete removal of free TOP1. We then digested purified DNA using DNaseI, and separated the resulting covalently trapped proteins using SDS-PAGE. After transferring to nitrocellulose membranes, we detected the proteins using anti-TOP1 antibody. We successfully detected TOP1 -DNA crosslinks using slot-blot. In future, we will analyze the TOP1cc repair mechanism after treatment with BP.