Title of Research:

16_PT01-01

Establishment of a comprehends test for identifying mutagenic chemical compounds and molecular mechanisms underlying mutagenesis

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Summary of Research:

(1) Creation of TK6 cells deficient in individual DNA repair proteins

The OECD countries have employed the human TK6 B lymphoid cells as a standard cell line for evaluating the genotoxicity of industrial chemical compounds. However, quick accurate repair of DNA damage induced by chemicals in DNA-repair proficient TK6 cells significantly limits the sensitive identification of mutagenic chemicals. To overcome this problem, we have disrupted genes encoding various DNA repair factors and shown the list of 119 DNA-repair mutants generated from TK6 cells in the web site (http://www.nihs.go.jp/dgm/tk6.html). The DNA-sensitivity profile of various DNA-repair mutants to given chemicals would allow for assessing the type of DNA lesion induced by them.

(2) Analysis of molecular mechanisms for mutagenesis induced by nucleoside analogs

Nucleoside analogs have been used for anti-viral and cancer therapies. We created a bioassay for comprehensively analyzing various mechanisms for mutagenesis caused by nucleoside analogs. This analysis will be published in *OncoTarget.*

(3) Establishment of micro nucleus (MN) assay using DNA repair deficient TK6 cells

We established XRCC1-deficient (*XRCC1*$^{-/-}$) TK6 cells, and conducted the MN assay using typical DNA damaging agents, such as X-rays and mitomycin-C. MN analyses with *XRCC1*$^{-/-}$ cells showed several times higher sensitivity than conventional MN analyses with wild-type TK6 cells.

(4) Discovery of an alternative excision repair of DNA damage induced by UV

We created *XPA*$^{-/-}$/*XRCC1*$^{-/-}$ TK6 cells, and uncover the functional overlap between nucleotide excision repair (NER) and base excision repair (BER). It has been believed that BER is unable to remove bulky DNA lesions induced by UV and crosslinking agents such as cisplatin. We found that XRCC1-dependent BER contributes to removal of the bulky DNA lesions in NER-deficient *XPA*$^{-/-}$ TK6 cells. Moreover, XRCC1-dependent BER is capable of removing the bulky DNA lesions on the DNA of mitochondria, where no functional NER is present.

Timeline:

March 1, 2016-

Topics:

2017 LRI Annual Meeting, “Improvement of MN test sensitivity in the detection of mutagenic chemicals by employing DNA damage repair mutant TK6 cells”

Publications:

Tsuda, M et al., The dominant role of proofreading exonuclease activity of replicative polymerase ε in cellular tolerance to cytarabine (Ara-C). *Oncotarget.* 8(20):33457-33474, 2017.