【Grant-in-Aid for Scientific Research(S)】
Integrated Science and Innovative Science (New multidisciplinary fields)

Title of Project : Mutants deficient in DNA repair pathways provide high throughput assays for genotoxicity of chemicals and contribute to development of *in silico* methods for predicting genotoxicity of chemicals from their structure

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Research Area : Radiation Biology, Toxicology, Molecular Biology
Keyword : Mutagen, toxic chemical compounds, ionizing radiation, Tox21 program

【Background of the Research】

Chemicals used industrially and commercially are required by law to be assessed for their genotoxic potential. However, all currently used bioassays, including the Ames test and the *in vitro* micronucleus assay, yield unacceptably large proportions of false-positive results. This limitation causes another problem. The ultimate goal of detecting toxicity associated with test chemical compounds is to develop a method for *in silico* prediction of toxicity from their chemical structures. Since this *in silico* prediction depends on the quality of the database, numerous false-positive results from currently used bioassays make development of reliable *in silico* prediction impossible.

To solve this problem, we have proposed new bioassays, where we evaluate genotoxicity using a Toxico-Genetic approach (Ref. 1). Previous bioassays employ only *wild-type* DNA-repair-proficient cells, while our new approach analyzes cellular responses to test chemicals by comparing between *wild-type* cells and isogenic DNA-repair-deficient mutant clones. If one of the mutants shows higher sensitivity to test chemicals than *wild-type* cells, it is concluded that their toxicity is certainly related with a relevant disabled DNA-repair enzyme, and is most likely to be attributable to DNA damage and resulting mutagenesis caused by these chemicals. It should be noted that in this Toxico-Genetic approach *wild-type* cells are served as a negative control to monitor false-positive results. Resulting high quality of data would be useful for establishing tools for the *in silico* prediction.

We have created more than 100 chicken DT40 clones deficient in individual DNA repair genes. We then proposed our bioassay of identifying mutagenic chemical compounds to scientists of National Toxicology Program (NTP) in USA. They agreed to let us join the Tox21 program, and we did the following preliminary experiment in National Institute of Health Chemical Genomics Center (NCGC) in 2008. We exposed five isogenic DT40 mutants and a *wild-type* control to 1405 chemical compounds, biological effects of which are well characterized by NTP.

【Purpose and Research Methods】

The purpose of our study is as follows:

(1) We optimize and validate the method noted above for the high throughput screening of mutagenic chemical compounds in NCGC. All data obtained in NCGC, including our data, must be deposited and open to public in the PubChem site.
(2) We develop a reliable *in silico* prediction tool by mining data deposited in the PubChem site.
(3) We create new bioassays to identify chemical compounds that damage mitochondria and endoplasmic reticulum (ER), and cause oxidative stress. To this end, we will generate DT40 mutants each deficient in the mitochondria quality control, ER stress response, or cellular response to oxidative stress.
(4) We analyze molecular mechanisms underlying the induction of mutations by ionizing radiation and various chemical compounds. To this end, we will disrupt genes involved in DNA repair and develop new phenotypic assays.

【Expected Research Achievements and Scientific Significance】

The Japanese Government will be able to improve methods to prevent the pollution of the environment by toxic chemical compounds.

【Publications Relevant to the Project】

(1) DNA Repair (Amst). 9: 1292-8, 2010

【Term of Project】 FY2011-2015

【Budget Allocation】 165,300 Thousand Yen

【Homepage Address and Other Contact Information】

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