【Grant-in-Aid for Scientific Research (S)】

Integrated Disciplines (Environmental Science)

Title of Project : Establishment of Novel Bioassays for in vivo Genotoxicity Prediction and Mechanism Characterization

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Research Project Number : 16H06306 Researcher Number : 60188191
Research Area : Environmental analyses and evaluation, Risk sciences of radiation and chemicals
Keyword : Toxicology, Toxic chemical compounds

【Purpose and Background of the Research】

Why you must establish a new bioassay to control genotoxic chemical products?

Every developed country requires companies to demonstrate the safety of chemical products before introducing to the market using methods specified by law. The methods of detecting genotoxicity were developed more than 30 years ago, and have the two weaknesses; (i) limited sensitivity and specificity (Ref. *Mutat. Res.* 588:47, 2005), and (ii) no information about the type of DNA lesions, such as DNA-strand breaks, hydrolysis of bases, and crosslink of nucleotides, generated by individual chemicals. The limited sensitivity is due to the usage of DNA-repair-proficient wild-type cells, which is capable of accurately repairing DNA damage caused by genotoxic chemical products.

Requirement of in silico genotoxicity test

A large number of chemicals are in the market without checking their genotoxicity if they had been sold before the law started controlling genotoxic products in 1973. In addition, genotoxicity tests are expensive. Thus, we need *in silico genotoxicity* tests, the computer-based prediction of genotoxicity from the structure of individual chemicals. To this end, you must have the study data that include minimum false-negative and false-positive data. The study data must also include information about the type of DNA lesions, the product of chemical reactions between DNA and genotoxic chemicals.

Purpose of our research

The aim of this research is to solve the above (i) and (ii) problems. To this end, we previously created a novel bioassay using the chicken DT40 cells, and tested its validity in National Institute of Health (NIH), US., using a golden-standard chemical library containing ~10,000 chemicals provided National Toxicology Program in US (see Publications Relevant to the Project).

【Research Methods】

(1) Development of new genotoxicity bioassays using the human TK6 cell line. The usage of the TK6 cell line is recommended by the OECD guideline. Our proposal is to include (a) TK6 mutant clones deficient in individual DNA-repair pathways generated by the gene-editing method, in addition to (b) wild-type TK6 cells for the genotoxicity test. If one of the mutant clones shows a more prominent response, such as higher micronuclei formation, to chemicals than do wild-type cells, we judge the chemicals as genotoxicity-positive. Bioassays with wild-type cells serve as a negative control, and would ensure the high specificity of our genotoxicity test.

(2) We will test the validity of the new bioassay in collaboration with NIH. US.

(3) We will explore novel mutagenic mechanisms.

【Expected Research Achievements and Scientific Significance】

Expected achievement is to significantly improve the current method of detecting mutagenic chemicals. Another achievement is to establish a new method of identifying the type of lesions induced by mutagenic chemicals. Identifying the type of lesions induced by a large number mutagenic chemicals would make the computer learn the quantitative relationship between the structure of chemicals and the type of DNA lesions induced by them.

【Publications Relevant to the Project】

・ Nishihara K, Huang R, Zhao J, Shahane SA, Witt SK, Smith-Roe SL, Tice RR, Takeda S, Xia M. (2016) Identification of genotoxic compounds using isogenic DNA repair deficient DT40 cell lines on a quantitative high throughput screening platform. Mutagenesis 31 (1): 69-81.

【Term of Project】

FY2016-2020

【Budget Allocation】

140,900 Thousand Yen

【Homepage Address and Other Contact Information】

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