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

Title of Project : Innovation of genotoxicity tests —DNA damage, Mutation, Chromosome—

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Research Area : New multidisciplinary fields
Keyword : Genotoxicity tests, DNA adductome, Mutation assay, Protein-complex analysis

【Purpose and Background of the Research】

Genotoxicity tests are essential for pre-marketing hazard assessment of newly-developed chemicals. However, the major genotoxicity tests have been developed several decades ago and are poor in prediction of carcinogenicity. Recent development of analytical equipments and cell biology allow us to make innovation in this area. In this study, we tried to develop innovative genotoxicity tests which focused on DNA damage, mutation and mechanism of chromosomal aberration.

【Research Methods】

We are going to develop three kinds of genotoxicity tests as described following.

① DNA adductome

This technology, we have described previously, enables us to detect known- or unknown-DNA damages directly by using LC/MS/MS. In this study, we tried to apply this method as an genotoxicity test. We will expose various chemicals to cultured cells, extract DNA, analyze their DNA damage by the DNA adductome method.

② Direct sequence of gene mutations

To evaluate the cancer risk of chemicals, many mutation assays have been developed, most using sophisticated genetic technologies. However, recent advance in DNA sequencing technology, at least in principal, enables us to detect chemically induced small frequency of gene mutations by direct DNA sequencing. For example, a leading-edge next generation DNA sequencers can analyze more than 100 Giga bases per analysis. Assume that a chemical induce one mutation per 1 Mega bases, 100,000 mutations are expected to be detected. In this way, applying a DNA sequencer to a mutation assay is very promising. We will develop a method for DNA-template preparation and data-treatment for this new mutation assay.

③ Evaluation of DNA damage-independent clastogenic mechanisms

Chromosomal aberration test is one of the important genotoxicity tests. However, the weak point of this test is having much false-positive results in terms of carcinogenicity. For example, caffeine and curcmin have a clastogenic potential at higher doses, but have no carcinogenicity. Chromosome is composed of DNA and various protein-complexes. Such protein-complexes are important for maintaining function and structure of chromosome. So that, the molecular targets of clastogen should be not only DNA but also these protein-complexes. In this study, we will carry out proteome analysis of important protein-complexes, and develop evaluation methods for stability of the protein-complexes against clastogens.

【Expected Research Achievements and Scientific Significance】

Proper strategy for genotoxicity-evaluation is becoming world-wide concern. These innovative genotoxicity tests will be useful tools for hazard assessment of chemicals and contribute to public health and environmental protection.

【Publications Relevant to the Project】

Matsuda, T. (2010) Anticipated Mutation Assay Using Single-molecule Real-time (SMRTTM) Sequencing Technology. Genes and Environment, 32, 21-24.

Chou, P. H. and Matsuda, T. et. al. (2010) Detection of lipid peroxidation-induced DNA adducts caused by 4-oxo-2(E)-nonenal and 4-oxo-2(E)-hexenal in human autopsy tissues. Chem Res Toxicol, 23, 1442-1448.

【Term of Project】 FY2011-2015

【Budget Allocation】 146, 400 Thousand Yen

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