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RESEARCH NEWS STORY

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Simple and Cost-Effective Reporter Assay for Evaluating Chemical-Induced Epigenetic Changes

Researchers developed a cell-based reporter assay that can quantify epigenetic changes induced by chemicals and potential carcinogens

Numerous widely used chemicals induce genetic and epigenetic alterations implicated in various diseases, including cancer. Safety assessment of potential carcinogens is necessary to minimize their hazardous impact. While genotoxicity assays are widely used to evaluate genetic changes, quantification of epigenetic changes requires advanced and expensive sequencing techniques. Researchers from Japan have developed a simple and cost-effective cell-based reporter assay that can quantify chemical-induced epigenetic effects, and enhance the safety evaluation of environmental chemicals.

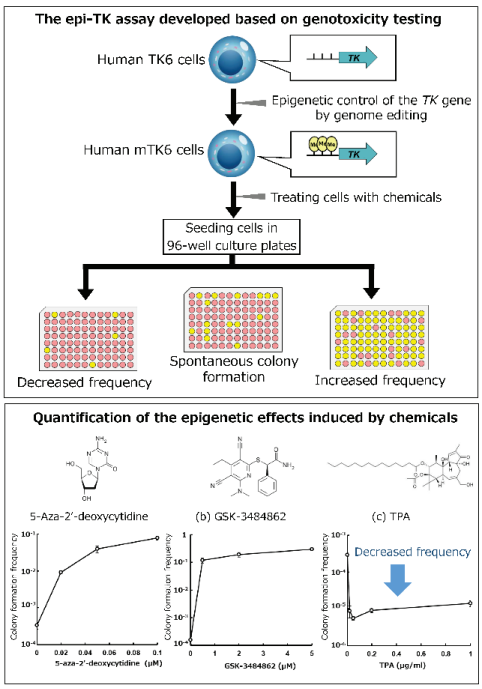

Image title: Researchers develop a cell-based reporter assay for quantification of chemical-induced epigenetic alterations
Image caption: Chemicals used in different applications induce genetic and epigenetic aberrations implicated in various diseases, including cancer. Researchers from Japan have developed a cell-based reporter assay for quantification of chemical-induced epigenetic alterations that can enhance the safety evaluation of potential carcinogens.
Image credit: Associate Professor Akira Sassa from Chiba University, Japan
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Chemicals used as food preservatives, flavoring agents, dyes, pesticides, cosmetics, cleaners, and other industrial materials are being increasingly recognized as a health hazard. Their rampant use has led to an increase in the prevalence of various chemical toxicity-induced diseases, including hormonal disruption, cancer, neurological disorders, skin conditions, and occupational poisoning. Numerous chemicals are known to trigger "carcinogenesis" or cancer development by exerting genotoxic effects (direct or indirect interference with DNA replication and damage repair processes resulting in mutations and chromosomal aberrations). Various *in vitro* genotoxicity assays help assess the interactions of potential carcinogens with DNA and elucidate their role in health and disease.

In addition to genotoxicity, epigenetic alterations, or reversible changes to DNA and chromatin (packaged DNA-protein complexes) have been implicated in chemically induced carcinogenesis. Typically, DNA methylation, the addition of methyl groups to DNA, silences gene expression. Conversely, acetylation (addition of acetyl groups) of histones, the proteins that bind DNA, opens up the chromatin structure, making DNA accessible for transcription. Such dynamic epigenetic alterations in DNA and histones regulate gene expression in a cell- and tissue-specific manner. Notably, environmental chemicals such as bisphenol A, arsenic, cadmium, benzene, pesticides, and other carcinogens have been reported to induce aberrant epigenetic changes in various diseases. Unraveling the mechanisms underlying chemical-induced epigenetic alterations can aid the safety assessment of environmental chemical compounds.

Cell-based assays that have been previously developed, detect epigenetic changes in a unidirectional manner (inactivation/reactivation of gene expression) based on the baseline status of the reporter gene and do not fully capture chemical-induced epigenetic alterations. A flexible, bidirectional assay needs to be developed to effectively detect diverse epigenetic impacts of chemical exposure in human cells.

To resolve this issue, Associate Professor Akira Sassa along with Professor Kiyoe Ura from the Graduate School of Science, Chiba University, Japan, and Manabu Yasui and Kei-ichi Sugiyama from the National Institute of Health Sciences, Japan, have developed a novel epi-genotoxicity assay to evaluate carcinogen-induced epigenetic changes.

Explaining the scientific rationale behind their work published in *Scientific Reports* on March 5, 2025, Assoc. Prof. Sassa says, *"In the field of genome biology, mastering epigenetic analysis techniques is both challenging and costly, making it difficult to use in the safety assessment of chemicals. This led us to consider developing a universally accessible method through collaborative research across academia, industry, and government. Elucidation of previously unknown epigenetic mechanisms of chemical carcinogenesis can aid safer chemical use worldwide, including in developing countries."*

Thymidine kinase (*TK*) gene mutation assay (TK assay) is a conventional *in vitro* genotoxicity test that detects mutations in the *TK* gene locus, an essential housekeeping gene expressed by all cell types. The researchers previously enhanced the TK assay and improved its detection sensitivity for the safety assessment of pharmaceutical, industrial, agricultural, and environmental chemicals with potential genotoxic and cytotoxic effects. In the current study, they have further developed an epi-TK reporter assay by site-specifically methylating the *TK* promoter region.

They assessed the capability of the epi-TK assay to reflect global epigenetic changes by quantifying "TK reversion" or the expansion of cells with a methylated (silenced) *TK* promoter following treatment with inhibitors of DNA methyl transferases (DNMTs) with well-characterized mechanisms of action to elucidate the potential of the system to reflect epigenetic changes.

Notably, treatment with DNMT inhibitors resulted in unmethylated sites within the TK promoter region and expansion of TK revertant colonies. Additionally, treatment with 12-O-tetradecanoylphorbol-13-acetate (TPA), a widely studied non-genotoxic seed oil-derived carcinogen, led to a significant decrease in TK revertant frequency and histone acetylation levels. The epi-TK assay could thus reflect epigenetic changes associated with both gene silencing and activation.

Contrary to advanced sequencing analysis that requires expensive reagents and instruments, along with specific technical and analytical expertise, the epi-TK reporter assay developed in this study offers a simpler, cost-effective, and quantitative approach to evaluate chemical epi-genomic toxicity.

Assoc. Prof. Sassa concludes by saying, *"Our research can enhance the understanding of the impact of chemicals on public health and disease prevention, thereby promoting safer management and use of chemicals. Understanding the relationship between environmental chemicals and diseases, and improving chemical safety evaluations, can aid the implementation of measures to reduce the exposure to harmful chemicals in both work and living environments."*

About Associate Professor Akira Sassa

Akira Sassa is an Associate Professor at the Graduate School of Science, Chiba University, Japan. He completed his doctoral studies at the Tokyo University of Pharmacy and Life Sciences. His research interests include genome biology and biochemistry, molecular mechanisms of genome instability and repair, and mechanisms of chromatin regulation. He is a council member of the Japanese Environmental Mutagen and Genome Society. Assoc. Prof. Sassa has authored several publications spanning genome biology, epigenetics, genotoxicity, and DNA mutations.

Reference:

Title of original paper: Dual-directional epi-genotoxicity assay for assessing chemically induced epigenetic effects utilizing the housekeeping *TK* gene
Authors: Haruto Yamada[1], Mizuki Odagiri[1], Keigo Yamakita[1], Aoi Chiba[1], Akiko Ukai[2], Manabu Yasui[2], Masamitsu Honma[2], Kei-ichi Sugiyama[2], Kiyoe Ura[1], and Akira Sassa[1]
Affiliations: [1]Department of Biology, Graduate School of Science, Chiba University, Chiba, Japan
[2]Division of Genome Safety Science, National Institute of Health Sciences, Kanagawa, Japan
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Contact:
Akira Sassa
Associate Professor,
Graduate School of Science, Chiba University, Japan
Email: a-sassa@chiba-u.jp

Public Relations Office, Chiba University
Address: 1-33 Yayoi, Inage, Chiba 263-8522 JAPAN
Email: koho-press@chiba-u.jp
Tel: +81-43-290-2018