LRI

Long-range Research Initiative

Annual Report 2017

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Japan Chemical Industry Association

Annual Report 2017

Title of Research:

13_S01-01-4

Development of the Novel Method with iPSCs to Assess Chemical Compounds for the Risk of CSC Induction

Principal Investigator:

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Collaborators:

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Yoshiaki Iwasaki, MD, PhD (Center of Healthcare, Okayama University)

Summary of Research:

The risk assessment for the cancer development caused by chemical compounds has long been made by mutagenicity or repeated dose toxicity study, by which it generally takes long time to get results. However, cancer is currently recognized consisting of heterogeneous population of cells and cancer stem cells (CSCs). This is altering the idea that a cancer tissue consists of a homogeneous population of clonal cells continuously proliferating due to the oncogenic mutations. It appears necessary to develop a novel method to identify chemical compounds that may induce cancer stem cells. In this study, we established a simple method to assess the risk of chemical compounds inducing CSCs employing mouse iPSCs, which have been reprogrammed from the embryonic fibroblast derived from a transgenic mouse carrying the Nanog promoter integrated with GFP gene. This method allows the assessment in 8 days by judging the increase of spheres and the fluorescence of GFP enhanced by Nanog gene expression. We evaluated 147 chemical compounds and 30 were judged as positive. Further, we tried to analyze the molecular mechanism inducing CSCs from miPSCs by microarray, CpG methylation, lipid component, bioinformatics and so on. As the results, the relationship of some chemokine and its receptors, some specific cytoplasmic signal transduction pathway, inflammatory factors were implied to be importantly involved. Collectively, it should be possible to find out the key relationships between the cancer risks and the mechanism of action of chemical compounds in the future.

Timeline:

March 1, 2012 – February 28, 2018

Topics:

Poster presentation entitled “Development of the Novel Method with iPSCs to Assess Chemical Compounds for the Risk of CSC Induction” at 2017 Annual Meeting of LRI.

Publications:

Nair N, Calle AS, Zahra MH, Prieto-Vila M, Oo AKK, Hurley L, Vaidyanath A, Seno A, Masuda J, Iwasaki Y, Tanaka H, Kasai T, Seno M. A cancer stem cell model as the point of origin of cancer-associated fibroblasts in tumor microenvironment. Sci Rep. 2017;7(1):6838.

Ninomiya T, Ohara T, Noma K, Katsura Y, Katsube R, Kashima H, Kato T, Tomono Y, Tazawa H, Kagawa S, Shirakawa Y, Kimura F, Chen L, Kasai T, Seno M, Matsukawa A, Fujiwara T. Iron depletion is a novel therapeutic strategy to target cancer stem cells. Oncotarget. 2017;8(58):98405-98416.

OoA, Calle AS, Nair N, Mahmud H, Vaidyanath A, Yamauchi J, Khayrani AC, Du J, Alam MJ, Seno A, Mizutani A, Murakami H, Iwasaki Y, Chen L, Kasai T, Seno M. Up-Regulation of PI 3-Kinases and the Activation of PI3K-Akt Signaling Pathway in Cancer Stem-Like Cells Through DNA Hypomethylation Mediated by the Cancer Microenvironment. Translational Oncology. 2018;11(3), 653-663.

Seno A, Seno M. Commonly expressed genes among cancer stem cells induced from hiPSCs and

obtained from cancer tissues or cell lines. Tumor and Microenvironment. 2018 *in press*.

Title of Research: 16_S01-01-2

Advanced system development for the hazard prediction and the environmental risk assessment/management of chemical substances

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Summary of Research: In this research, collaboration between AIST and Toyohashi University of Technology will develop an advanced system to enable risk assessment management of unconfirmed existing chemical substances and new substances without hazard information faced by Chemical Industry Association. Toward the goal of designated research, the following research outputs were obtained. 1) MeRAM Ver.2.0 as the latest released version (Japanese version & English version) were open for public; (2) added a function to MeRAM that can automatically export the built-in data of MeRAM according to various purposes of users; (3) In order to be used by environmental administration, a function that can automatically launch KATE from the interface of MeRAM, and then can automatically acquire the predicted value of KATE and use it for risk assessment; Although the release of the MeRAM latest version equipped with the functions of 2) and 3) is not yet available, it was distributed to many participants at seminars of the Ministry of the Environment and workshops organized by AIST; (4) Started to develop our own methodology for predicting the toxicity of chemical substances from October of last year. Based on various fingerprint information (structure and physical information) of chemical substances and toxicity information, toxicity estimation by machine learning but not using QSAR equations is under developed.

Timeline: From March 2017 to February 2018

Topics: None

Publications:

1) (Paper Publication) Institution magazine "Toxicity Inquiry Box" of the Safety Assessment Committee, 2017: "Ecological risk assessment of chemical substances in water environment".
2) (Presentation at Domestic Meeting) The 26th Environmental Chemistry Debate (7-9 June 2017), Shizuoka Convention Center, Shizioka, “IT solution for Cumbersome Ecological Risk Assessment and Management of Chemicals: AIST-MeRAM”.
3) (Invited Speech at Domestic Workshop) The 26th Environmental Chemistry Debate (7-9 June 2017), Shizuoka Convention Center, Shizioka, "Open Science in Environmental Chemistry" Why do not open your own data: Potential of using open data."
4) (Presentation at Domestic Meeting) The 20th Water Environment Symposium (26-27 September 2017), Wakayama University, “Hierarchical ecological risk assessment and management of chemical substances using MeRAM”.
5) (Presentation at Domestic Meeting) The Tougou-no-hi-no Symposium (4-5 October 2017), Tokyo University, “Attempt to utilize integrated database for environmental risk assessment and risk communication of chemical substances”.
6) (Presentation at Domestic Meeting) The 30th Annual Meeting of Japan Risk Research (27-29 October 2017), Shiga University, “Risk assessment of toxicity deficient substances by linking MeRAM and KATE”.
7) (Invited Speech at Domestic Workshop) Environmental Science Seminar hosted by Ministry of the Environment Seminar on chemical substance environment survey (15-16 January 2018), Tokyo, " What MeRAM can do."
8) (Invited Speech at Domestic Workshop) MeRAM Workshop hosted by AIST (23 January 2018),

Tokyo.

Title of Research: 16_S01-01-2

Advanced system development for the hazard prediction and the environmental risk assessment/management of chemical substances
Subtitle: Development of the sophisticated basis tool environmental hazard prediction

Principal Investigator:
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Tsubasa Ishikawa (Graduate student, Toyohashi Univ. Tech.)

Summary of Research:
In this fiscal year (Fiscal 2017) research, we designed external file specifications for data import to PEACH(Prediction of Environmental Affect of Chemicals) with the aim of utilizing external data, and implemented the import function of external data utilizing the chemical structure dictionary. For the chemical structure dictionary tool developed in the previous year, we expanded the data import function and revised the user-interface for strengthening cooperation with MeRAM. In addition, we refined the estimation model of water solubility parameter logS by atomic fragment method. We developed an estimation model of vapor pressure too. On the other hand, for the short-term toxicity (96h-LC50) against fish, we developed alternative prediction models based on quantitative activity-activity relationships (QAAR) for the toxicity tests with different fish species. The results show that the QAAR models would be very useful for prediction of missing data.

Timeline:
1st Mar. 2016 – 28th Feb. 2017

Topics:
Poster presentatikon at ICCA-LRI International Workshop 2016, Awaji, Jun., 2016

Publications:
1) Tsubasa Ishikawa, Tetsuo Katsuragi, Bin-Le Lin, Yoshimasa Takahashi, Studies on complementation of missing data of fish toxicity by Quantitative Activity-Activity Relationship, 26th Symposium on Environmental Chemistry, Shizuoka, 2017.
2) Taku Izumihara, Tetsuo Katsuragi, Bin-Le Lin, Yoshimasa Takahashi, Construction of Chemical Structure Dictionary for MeRAM, 26th Symposium on Environmental Chemistry, Shizuoka, 2017.
3) Mitsuru Sato, Dai Furukawa, Tetsuo Katsuragi, Yoshimasa Takahashi, Prediction of Aqueous-Solubility Using an Atomic Group Contribution Method, 26th Symposium on Environmental Chemistry, Shizuoka, 2017.
4) Yoshimasa TAKAHASHI and Ryota KIKUCHI, Algal toxicity prediction of chemicals using TFS-PLS method in conjunction with active QSAR modeling, Abstracts of the 53rd Congress of the European Societies of Toxicology (EUROTOX) Bratislava, Slovakia, 10th–13th September, 2017. ; *Toxicology Letters* 280S (2017) S316.
5) Yoshitaka Inagaki, Tomoya Yamazaki, Yoshimasa Takahashi, “Development of a desk-top tool ToxCalc for eco-toxicity prediction of chemicals”, The 43rd Symposium on Structure-Activity Relationships, Tsuchiura, Nov., 2017.

*Title of Research:*16_S02-02-2

Prediction method (Amino acid Derivative Reactivity Assay: ADRA) for skin sensitization using novel lysine and cysteine derivatives

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Summary of Research:

In this study, we performed the validation study so that the alternative method for skin sensitization (ADRA) is adopted as OECD test guideline.

Part1)

For the four participating laboratories, the two training sessions were held, and the two confirmation tests (Pre-training test and Training test) were performed because the lead laboratory confirmed that the assay technique was correctly transferred for the participating laboratories.

Compared with cell-based *in vitro* test methods, DPRA is an easy-to-use test method and exhibits excellent predictive capacity. Nevertheless, the DPRA test method has significant limitations:

- One of the nucleophilic reagents, cysteine peptide, is easily oxidized rendering some test results invalid, unreliable or difficult to interpret.
- It requires relatively high concentrations of the test chemical, making it unsuitable for evaluating poorly soluble chemicals.
- It requires a relatively large quantity of both test chemicals and nucleophilic reagents to perform.
- Hydrophobic test chemicals tend to precipitate easily in the reaction solution.

ADRA, an *in chemico* test which is also based on protein reactivity, does not have these limitations thanks to the use of highly sensitive nucleophilic reagents, which allows reagent solutions to be prepared at test chemical concentrations just 1% of those required in DPRA.

Following a modular approach applying the ECVAM principles on test validity (OECD Series on Testing and Assessment, Number 34, 2005, Hartung *et al.* 2004), the Validation Management Team (VMT) empirically evaluated modules one through four (test definition, within-laboratory reproducibility, transferability, and between-laboratory reproducibility), and used these results also to evaluate modules five and six (predictive capacity and applicability domain).

During a preliminary training phase of the ADRA validation study using ten test chemicals, the results obtained by the four participating laboratories were 100% concordant with the lead laboratory. During the subsequent transferability phase of the study, three of the four participating laboratories were 100% (10/10) concordant and the fourth laboratory was 90% (9/10) concordant with the lead laboratory. The ADRA VMT considers these results to have confirmed that the ADRA test method is easily transferred to naïve laboratories.

During Phase I of the Study Plan, each of the four participating laboratories performed three test runs of identical sets of 10 coded test chemicals to evaluate within-laboratory reproducibility. The results, based on concordance, were 100% (10/10), 100% (10/10), 100% (7/7), and 90% (9/10). When the results from the 10 test chemicals of Phase I were combined with those of Phase II, in which each participating laboratory performed one test run of identical sets of an additional 30 test chemicals, between-laboratory reproducibility for 40 test chemicals was 92%. The VMT considers

this to satisfy standards for both within- and between-laboratory reproducibility for this class of test method.
Moreover, a review of predictive capacity relative to LLNA based on the results of Phases I and II yielded a sensitivity of 81%, specificity of 98%, and accuracy of 86%. The VMT therefore considers ADRA to be an *in chemico* method with sufficient specificity, sensitivity, and predictive capacity for regulatory acceptance.

Part 2)

During a recent validation study conducted at multiple laboratories as part of the process to include ADRA in an existing OECD test guideline, one of the nucleophilic reagents used in ADRA—*N*-(2-(1-naphthyl)acetyl)-*L*-cysteine (NAC)—was found to be susceptible to oxidation in much the same manner that the cysteine peptide used in DPRA was. Due to this, we undertook a study to clarify the cause of the promotion of NAC oxidation.

In general, cysteine and other chemicals that have thiol groups are known to be oxidized in the presence of minute quantities of metal ions. When Fe and Cu ions were added to the ADRA reaction solution, the Cu ions facilitated NAC oxidation significantly. When 0.25 µM of EDTA was added in the presence of Cu ions, NAC oxidation was suppressed. Based on this, we predicted that the addition of EDTA to the NAC stock solution would suppress NAC oxidation. Next, we tested 82 chemicals used in developing ADRA to determine whether or not EDTA affects ADRA's ability to predict sensitization. The results showed that the addition of EDTA has virtually no effect on the reactivity of NAC with a test chemical and yielded an accuracy of 87% for predictions of skin sensitization, which was roughly the same as ADRA.

Timeline: March 1, 2017- (it continues from March 1, 2016)
Topics: none
Publications:

1. Presented at the 44th The Japanese Society of Toxicology (2017.7, Yokohama) 「Multicenter Validation Study of Novel *in chemico* Skin Sensitization Assay (ADRA): 1st Report」
   Atsushi Ono, Shin-ichi Watanabe, Tsunetsugu Sugawara, Koji Wakabayashi, Yu Tahara, Nobuyuki Horie, Keiichi Fujimoto, Kei Kusakari, Yoshihiko Kurokawa, Takashi Sozu, Takuto Nakayama, Takeru Kusao, Tsuyoshi Kawakami, Kohichi Kojima, Hajime Kojima, Jon Richmond, Nicole Kleinstreuer, Bae-Hwa Kim, Yusuke Yamamoto, Masaharu Fujita, and Toshihiko Kasahara
2. Presented at the 10th World Congress on Alternatives and Animals in the Life Sciences (WC10) (2017.8) (Seattle, WA, USA) 「Phase-1 of the validation study of Amino acid Derivative Reactivity Assay (ADRA) : a novel *in chemico* alternative test method of skin sensitization.」
   Atsushi Ono, Shin-ichi Watanabe, Tsunetsugu Sugawara, Koji Wakabayashi, Yu Tahara, Nobuyuki Horie, Keiichi Fujimoto, Kei Kusakari, Yoshihiko Kurokawa, Takashi Sozu, Takuto Nakayama, Takeru Kusao, Tsuyoshi Kawakami, Kohichi Kojima, Hajime Kojima, Jon Richmond, Nicole Kleinstreuer, Bae-Hwa Kim, Yusuke Yamamoto, Masaharu Fujita, and Toshihiko Kasahara
3. Presented at the 30th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments」 (2017.11, Tokyo) 「Multi-laboratory validation study of ADRA as novel in chemico alternative test method for skin sensitization: 2nd report」 Atsushi Ono, Shin-ichi Watanabe, Tsunetsugu Sugawara, Koji Wakabayashi, Yu Tahara, Nobuyuki Horie, Keiichi Fujimoto, Kei Kusakari, Yoshihiko Kurokawa, Takashi Sozu, Takuto Nakayama, Takeru Kusao, Tsuyoshi Kawakami, Kohichi Kojima, Hajime Kojima, Jon Richmond, Nicole Kleinstreuer, Bae-Hwa Kim, Yusuke Yamamoto, Masaharu Fujita, and Toshihiko Kasahara
4. The Cause of and Countermeasures for Oxidation of the Cysteine-Derived Reagent Used in the Amino acid Derivative Reactivity Assay. (in submission to Journal of Applied Toxicology) Masaharu Fujita, Yusuke Yamamoto, Shinichi Watanabe, Tsunetsugu Sugawara, Koji Wakabayashi, Yu Tahara, Nobuyuki Horie, Keiichi Fujimoto, Kei Kusakari, Yoshihiko Kurokawa, Tsuyoshi Kawakami, Kohichi Kojima, Hajime Kojima, Atsushi Ono, Yasuhiro Katsuoka, Hideto Tanabe, Hiroshi Yokoyama, and Toshihiko Kasahara.

Title of Research:

15_S03-01-3

Establishment of in vitro evaluation system for the prediction of respiratory sensitizing potential of chemicals

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Summary of Research:

Recently, several in vitro assays to predict the respiratory sensitizing potential of chemicals have been developed, because the use of animal models in safety testing of chemicals is significantly limited. However, these alternative methods cannot distinguish chemical respiratory sensitizers and skin sensitizers, although the risk management systems for them are quite different. Therefore, in the present study, we aim at developing a novel in vitro assay, which can discriminate chemical respiratory sensitizers from skin sensitizers by taking advantage of the fundamental differences between their modes of function; development of helper T (Th) 2 immune responses, which is critically important for respiratory sensitization.

In this year, we have established an immunocompetent 3D co-culture system of human upper airway, which consists of human airway epithelial cells, immature dendritic cells (DCs) derived from human peripheral blood $CD14^+$ monocytes, and human fibroblast cells cultured in individual scaffolds. Then, these scaffolds were assembled into a 3D multi-cell tissue model, which more closely mimics the in vivo situation. Then, three typical chemicals for respiratory and skin sensitizers were added on the 3D co-culture system. The present results indicate that this 3D co-culture system can predict the respiratory sensitizing potential of chemicals by means of enhanced expression of key costimulatory molecule OX40L, which is important for Th2 differentiation, as markers in DCs (1). Moreover, to improve the versatility, we have next utilized iPS cell-derived DCs based on published protocols, although this efficacy is not good. Therefore, we are currently trying to establish a novel method to generate infinitely growing myeloid progenitors by stimulation with various cytokines and incorporation of genes related to cell cycle and survival. In addition, we are also trying to develop a novel 3DC/T system, which consists of sensitized DCs and allogenic naive $CD4^+$ T cells. In this 3DC/T system, IL-4 up-regulation could be the best marker for the prediction of respiratory sensitizing potential of chemicals.

Timeline:

March 1, 2017-Feberary 28, 2018

Topics:

The 5th Annual Meeting of The Japan Chemical Industry Association LRI, poster presentation “Establishment of a novel in vitro evaluation system for the prediction of respiratory sensitizing potential of chemicals” (The Tokyo Shoken Building, August 25, 2017)

Publications:

1. Mizoguchi I, Ohashi M, Chiba Y, Hasegawa H, Xu M, Owaki T, Yoshimoto T. Prediction of chemical respiratory and contact sensitizers by OX40L expression in dendritic cellsusing a novel 3D coculture system. *Front Immunol*. 8**,** 929, 2017 (IF=6.429).
2. Ohashi M, Mizoguchi I, Chiba Y, Hasegawa H, Xu M, Yoshimoto T. Prediction of chemical respiratory and contact sensitizers by OX40L expression in dendritic cells using a novel 3D co-culture system. The 46th Annual Meeting of The Japanese Society for Immunology, oral poster presentation in English (Sendai, December 12-14, 2017)

Title of Research:

17_S05-01

Development of Novel Analytical System of Biological Responses to Chemicals by Deep Phenotyping Method

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Summary of Research:

Today, organisms are always exposed to chemicals in every situation to some extent. Wide variety of chemicals exist around us and the chemicals affect organisms not one by one but simultaneously like a complex. The fact prompts us to care “multiple exposure”. To achieve the appropriate and correct prediction of multiple exposure of chemicals, it is necessary to comprehend the effects of chemicals even if those are hidden and difficult to be detected. In this study, we tackle with the theme by omic approaches with innovative techniques in both data acquisition and data analysis method.

This study consists of two pillars: (1) a novel acquisition method of proteome profile data by 2-dimensional electrophoresis (2DE) and (2) a new analysis method of profile data. In the 5th term, we carried out fine-tuning of these two. As for the novel data analysis method, aiming the establishment and the operation, performance tests were conducted by employing data obtained from public database (GEO dataset, CMap, etc). The data were mainly transcriptome data and were subjected to the novel method, “Orthogonal Liner Separation Analysis”, OLSA, followed by literary survey, gene ontology analysis, gathering information from compounds database (PubChem, Drugbank, etc.) and so on. The results indicate the following: (a) OLSA enables the separation and the extraction of cellular responses as vectors, (b) a vector generated by OLSA may be possible to be added or subtracted the others, and (c) the prediction by OLSA was confirmed experimentally to some extent. Of note, (b) is quite curious since it supports our hypothesis that cellular responses (, at least a part of them,) can be linearly separated and described with the separated vectors, which is essential in the prediction and evaluation of multiple exposure. Although, as for 2DE, a problem existing in fluorescent dye due to contaminants delayed the progress, the problem was already solved. Therefore, we believe our progress in the establishment of the prediction and the evaluation method of multiple exposure.

Timeline:

1/4/2017-

Topics:

2017 LRI annual meeting, “Development of Novel Analytical System of Biological Responses to Chemicals by Deep Phenotyping Method”

Publications:

Nothing

Title of Research:

13_PT01-01

Development of in vivo fluorescent imaging of neuronal differentiation in zebrafish for developmental neurotoxicity testing

Principal Investigator:

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Collaborators:

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Summary of Research:

The developing brain is extremely sensitive to many chemicals. Exposure to neurotoxicants during development has been implicated in various neuropsychiatric and neurological disorders, including autism spectrum disorder, attention deficit hyperactive disorder, schizophrenia, Parkinson's disease, and Alzheimer's disease. Although rodents have been widely used for developmental neurotoxicity testing, experiments using large numbers of rodents are time-consuming, expensive, and raise ethical concerns. Using alternative non-mammalian animal models may relieve some of these pressures by allowing testing of large numbers of subjects while reducing expenses and minimizing the use of mammalian subjects. Recently, zebrafish has emerged as an alternative non-mammalian animal model that allows testing of large numbers of subjects while reducing expenses and minimizing the use of mammalian subjects.

In this study, we have developed a transgenic zebrafish line whose neurons, astrocytes, and oligodendrocytes can be visualized using fluorescence stereomicroscope. Using the zebrafish, we evaluated the developmental neurotoxicity of 10 positive and 3 negative control chemicals. The neuronal differentiation of the transgenic zebrafish was significantly affected by 9 out of the 10 positive control chemicals. The 3 negative control chemicals did not show any significant effects on the neuronal differentiation. Hierarchical clustering of the 13 chemicals based on the fluorescent signals revealed the similarity among valproic acid, trichostatin A, and carbamazepine and the similarity between nicotine and chlorpyrifos. Valproic acid, trichostatin A, and carbamazepine are histone deacetylase inhibitors. Nicotine and chlorpyrifos can disrupt acetylcholine signaling. These results suggest that *in vivo* fluorescent imaging of neuronal differentiation in the transgenic zebrafish developed in this study can be a useful tool not only to detect the developmental neurotoxicity of various chemicals but also to reveal the adverse outcome pathways.

Timeline:

Nov 2013 ~ Feb 2018

Topics:

Presented at TEST SMART DNT4 (2014) and ICCA-LRI and NIHS workshop（2016）

Publications:

1) Nishimura Y, Murakami S, Ashikawa Y, Sasagawa S, Umemoto N, Shimada Y, Tanaka T: **Zebrafish as a systems toxicology model for developmental neurotoxicity testing**. *Congenital anomalies* 2015; **55**(1):1-16.
2) Nishimura Y, Okabe S, Sasagawa S, Murakami S, Ashikawa Y, Yuge M, Kawaguchi K, Kawase R, Tanaka T: **Pharmacological profiling of zebrafish behavior using chemical and genetic classification of sleep-wake modifiers**. *Frontiers in pharmacology* 2015; **6**:257.
3) Nishimura Y, Inoue A, Sasagawa S, Koiwa J, Kawaguchi K, Kawase R, Maruyama T, Kim S, Tanaka T: **Using zebrafish in systems toxicology for developmental toxicity testing**. *Congenital anomalies* 2016; **56**(1):18-27.

Title of Research:

15_PT01-01

Development of new *in vivo* screening method to validate the low-dose effects induced by estrogenic chemicals using estrogen reporter mice

Principal Investigator:

Tsuyoshi Nakanishi (Laboratory of Hygienic Chemistry and Molecular Toxicology, Gifu Pharmaceutical University)

Collaborators:

Hisamitsu Nagase (Laboratory of Hygienic Chemistry and Molecular Toxicology, Gifu Pharmaceutical University)

Summary of Research:

Background: The uterotrophic bioassay has been proposed as a short-term *in vivo* screening test to detect the estrogenic properties of potentially endocrine disrupting chemicals by the Organisation for Economic Co-operation and Development (OECD). Although the test is already standardized as a OECD test guideline (Test No.440), there is a possibility that the test might overlook a certain estrogenic properties which has no effect to the uterus. On the other hand, we recently generated a new transgenic mouse carrying “E-Rep” reporter gene which consisted of the luciferase (Luc) under the control of estrogen-responsive elements coupled to a minimal promoter. E-Rep mouse is a potential powerful tool for assessing the kinetics of gene activation by estrogenic properties *in vivo*. In the 4th term, we have established a provisional protocol for the novel *in vivo* screening test, which employs bioluminescence imaging in the whole body as the end point for estrogenic action of test chemicals.

Objective: In the current term, we verified the validity of the provisional protocol by confirming whether the estrogen-induced bioluminescence imaging in the whole body reflects the estrogenic action in each organ. In addition, using the bone tissue-specific selective estrogen receptor modulators (SERM), which have little effect on the uterus, we confirmed whether our novel *in vivo* screening test have an advantage over the uterotrophic bioassay.

Methods: The experimental protocols of the new assay using E-Rep mice are based on the uterotrophic bioassay with some modifications. Briefly, E-Rep female mice (8 weeks of age) were ovariectomized and then fed phytoestrogen-free purified diet during the experimental period. After 7 days of ovariectomy, the mice were orally gaveged with 17α-ethinylestradiol (EE) or SERMs for 7 consecutive days. The Luc activity was monitored in living animals with IVIS imaging at Day 1, 2, 4 and 7. After last detection of *in vivo* bioluminescence imaging, mice were dissected and measured uterine weight in accordance with the uterotrophic bioassay. For measurement of daily Luc activity induced by EE in each organ, EE-gaveged mice were dissected and removed each organ at Day 1, 2, 4 and 7.

Results: According to measurement of daily Luc activity in each organ, it was confirmed that the EE-induced bioluminescence imaging in the whole body reflects its estrogenic action in each organ. Furthermore In the experiment using the bone tissue-specific SERMs, our novel *in vivo* screening test could determine the SERMs as positive estrogens, whereas the uterotrophic bioassay could not determine a SERM even at a dose which show sufficient estrogen activity in the bone tissue.

Conclusion: Our novel *in vivo* screening test using E-Rep mice with *in vivo* bioluminescence imaging is an excellent method that can detect SERMs that cannot be detected by the uterotrophic bioassay.

Timeline: April, 2017 – March, 2018

Topics:

1) Dr. Tsuyoshi Nakanishi was awarded the 4th JCIA-LRI award for the research theme "Comprehensive research on reproductive toxicity of nuclear receptor-activated hazards and establishment of its evaluation system".

Publications:

1) Nishioka Y, Tamai K, Onda M, Hiromori Y, Kimura T, Hu J, Nagase H, Nakanishi T, Potential interference of oil vehicles on genital tubercle development during the fetal period in ICR mice, ***Biol. Pharm. Bull.***, 41:266-271 (2018)

Title of Research:

16_PT01-01

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

Principal Investigator:

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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.

Title of Research:

16_PT01-02-2

Construction of prediction models for toxicities induced by drugs and chemicals based on chemical structures and AOP

Principal Investigator:

Yoshihiro Uesawa

Summary of Research:

Quantitative structure–activity relationship (QSAR) analysis uses structural, quantum chemical, and physicochemical features calculated from molecular geometry as explanatory variables predicting physiological activity. Recently, deep learning based on advanced artificial neural networks has demonstrated excellent performance in the discipline of QSAR research. While it has properties of feature representation learning that directly calculate feature values from molecular structure, the use of this potential function is limited in QSAR modeling. The present study applied this function of feature representation learning to QSAR analysis by incorporating 360° images of molecular conformations into deep learning. Accordingly, we successfully constructed a highly versatile identification model for chemical compounds that induce mitochondrial membrane potential disruption with the external validation area under the receiver operating characteristic curve of ≥0.9.

Timeline:

March, 2017 – February, 2018

Topics:

Uesawa, Y., inventor; Meiji Pharmaceutical University, assignee. Prediction systems, prediction methods, prediction programs, learning model input data generation systems, and learning model input data generation programs. Pending Japanese patent application 2017-129823. 2017.

Publications:

1. Yuki Asako, Yoshihiro Uesawa, “High-Performance Prediction of Agonists on Human Estrogen Receptor Based on Chemical Structures”, Molecules, 23;22(4). pii: E675, 2017
2. Adverse effect predictions based on computational toxicology techniques and large-scale databases, 137th annual meeting of the pharmaceutical society of Japan, symposium, Sendai Japan (invited), 2017/3
3. “Application of JADER in palliative pharmacy care”, The 11th Annual Meeting of Japanese Society for Pharmaceutical Palliative Care and Sciences, symposium, Sapporo, Japan(invited), 2017/6
4. “Drug toxicity predictions based on databases for adverse effects and chemical structures”, The 24th HAB annual meeting, Tokyo, Japan(invited), 2017/6
5. “Analyses of adverse outcome pathways based on artificial intelligence”, The 44th Annual Meeting of the Japanese Society of Toxicology, Yokohama, Japan(invited)2017/7
6. “Construction of prediction models for toxicity reactions based on AOP and chemical structures”, Japan Chemical Industry Association LRI symposium, Tokyo, Japan(invited) 2017/8
7. “Application of AI technology in liver toxicity prediction via adverse outcome pathways”, Informatics In Biology, Medicine and Pharmacology2017, Sapporo, Japan(invited) 2017/9/
8. “The future of toxicity prediction developed by artificial intelligence”, 196th R&D Conference, Tokyo, Japan (invited)2017/12
9. Yurie Yoshida, Ryota Higuchi, Jun Teraoka, Yuhei Mashiyama, Hajime Kagaya, Yoshihiro Uesawa, “Comprehensive analysis of drug-induced thrombocytopenia in Japanese Adverse Drug Event Report database with chemical-structural information”, 137th annual meeting of the pharmaceutical society of Japan, , Sendai Japan, 2017/3
10. Yuhei Mashiyama, Yurie Yoshida, Ryota Higuchi, Jun Teraoka, Hajime Kagaya, Yoshihiro Uesawa, “Comprehensive analysis of drug-induced erythema exudativum multiforme major in Japanese Adverse Drug Event Report database with chemical-structural information”, 137th

annual meeting of the pharmaceutical society of Japan, , Sendai Japan, 2017/3

Title of Research:
17_PT01-01
Chip device for in vitro systemic toxicology

Principal Investigator:
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Summary of Research:
The purpose of this study is to fabricate microdevices for *in vitro* drug testing to evaluate systemic toxicity of chemicals, so-called Body on a chip. Unlike previous typical animal testing alternatives, our device may possibly enable to replicate *in vivo* kinetics and interactions between several tissues and organs including the skin, the small intestine, and the lung for drug absorption, liver and myocardial tissues for drug metabolism and exposure.

In the first year of the project, we engineered skin and small intestine tissues as cellular layers and liver tissues as spherical aggregates. Furthermore, a microdevice where these tissues were integrated and connected with micro fluidic channels was fabricated. To circulate culture medium in the microdevice, a seesaw-like bioreactor was designed, with which culture medium were perfused in the microdevice without any external pump and air pressure supply. We demonstrated that multiple tissues were formed and cultured in the microdevice on the bioreactor. Our next step is to demonstrate that our approach can be used to evaluate absorption dynamics of chemical substances passing through the skin and small intestine tissues and metabolic dynamics with the liver tissues.

Timeline:
May 1, 2017-

Topics:
Junji Fukuda, “Chip device for in vitro systemic toxicology”, JCIA LRI Research Conference, Tokyo, Japan, Aug 2017

Publications:
Keito Iseki and Junji Fukuda, “Development of drug screening device using seesaw type bioreactor”, 30th The Japanese Society for Alternative to Animal Experiences, Tokyo, Japan, Nov 2017

Title of Research:

17_PT01-02

Development of novel screening system to predict the effects of environmental factors in each organ by using cell-free circulating DNA in serum

Subtitle: Advanced system for prediction of hazards from environmental factors in fetus using cell free fetal DAN in mother's blood

Principal Investigator:

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Yusuke Takatsuru (Assistant Professor, Department of Integrative Physiology, Graduate School of Medicine, Gunma University)
Takahiko Katoh (Professor, Department of Public health, Graduate School of Medicine, Kumamoto University))

Summary of Research:

To prevent the several adverse effects induced by environmental factors including chemicals, it is very important to develop a novel screening system for each organ. Moreover, the system should be low invasiveness and simple method to obtain the results immediately. In this study, to develop the system, we have focused on the differences of epigenetic status in each organ-derived genome DNA and utilized cell free circulating DNA(cfDNA) / cell free fetal circulating DNA(cffDNA) to predict and evaluate the effects of environmental factors in whole organs. This advanced system also have possibilities to predict the effects of the factors in fetus using mother's serum.
Through this term, first, we performed whole genome bisulfite sequence (WGBS) to explore the organ specific markers in brain, liver, and heart from embryonic day14.5 (E14.5) fetuses. These data were combined with the others of dams and E12.5 fetuses from Gene Expression Omnibus (GEO), and we picked some candidates out for each organ. To investigate whether these candidates are useful for the system or not, we have evaluated these using the dummy of cfDNA derived from fetal tissues. In addition, we have produced organ specific injured-model mice for brain, liver, kidney, and pancreas to examine the specificities of the candidates for these organs.

Timeline:

March 1, 2017 -

Topics:

Poster presentation at 2017th JCIA LRI Annual Workshop, title: Development of novel screening system to predict the effects of environmental factors in each organ by using cell-free circulating DNA in serum

Publications:

Title of Research:

17_PT02-01

Establishment of mechanism based assay protocol for hasard and carcinogenic risk of carbon based nanomaterials

Principal Investigator:

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Summary of Research:

For the purpose of evaluating the toxicity and carcinogenicity of nanomaterials, particularly carbon nanotubes, we are establishing a method that is suitable for risk assessment and is far cheaper than whole body inhalation exposure. Whole body inhalation exposure requires specialized equipment and is exceedingly expensive, making it impossible to use except for a very small number of research institutes. Consequently, whole body inhalation testing can not be used to test the large number of inhalable carcinogens being produced. The method we are establishing for toxicity and carcinogenicity testing uses Trans-tracheal Intra-pulmonary Spraying (TIPS). In contrast to whole body inhalation exposure, TIPS can be used by many hundreds of researchers, making it possible to test all of the carcinogens workers and users are being exposed to. To examine the usefulness of the TIPS methods for practical use, we are currently testing MWCNT-7 (a known carcinogen composed of 40 layers of graphene) and Double-Walled Carbon Nanotubes (DWCNT) (2 layers). Method: Based on preliminary results using another DWCNT available from Sigma-Aldrich, 10 week old male F344 rats were treated with the test DWCNT or MWCNT-7 suspended in a vehicle containing 0.5% PF68 (a polyoxyethylene-polyoxypropylene block copolymer) on alternate days for 15 days (8 times in total) at final doses of 0.25 and 0.5mg/rat of the CNTs. Rats were sacrificed at 3 and 8 weeks after the start of administration, and the lung, tracheal lung lavage fluid (BALF), and supernatant and cell pellet of the thoracic lavage fluid (PLF) were analyzed.

Results: An inflammatory reaction was induced by DWCNT but the reaction was markedly less in rats administered DWCNT compared to rats administered MWCNT-7.

Timeline:

March, 2017 – February, 2018

Topics:

The 5th Research Achievement Presentation Meeting
Date:Augst 25th, 2017
Title: Establishment of mechanism based assay protocol for hasard and carcinogenic risk of carbon based nanomaterials

Publications:

None

Title of Research:

12_PT03-01-5

Development of high sensitivity in vitro assessment system of chemical-mediated hypersensitivity by using serine protease inhibitor-deficient cells
Subtitle: Analysis of in vitro assessment for respiratory allergy

Principal Investigator:

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Summary of Research:

The allergic disorders triggered by various chemicals are separated into two types of diseases, IgE-dependent immediate allergy and IgE-independent chronic allergy. Mast cells, basophils and eosinophils act as the effector cells in both allergic reactions. In particular, basophils and eosinophils rapidly infiltrate into respiratory tissue, and cause airway hyper-responsiveness and airway obstruction. These cells secrete abundant serine proteases as well as chemical mediators and cytokines. Serine proteases, such as elastases, disrupt the basement membrane, leading to the infiltration of inflammatory cells. The serine proteases are repressed by the serine protease inhibitors. Because we found that basophils and eosinophils lacking a secretory leukoprotease inhibitor SLPI produced more cytokines than wild type cells after stimulation with IgE or LPS. Therefore, we have planed to establish the highly sensitive cell line by the deletion of serine protease inhibitors using a human basophilic cell line, KU812-F. Because KU812-F expresses high levels of Serpin b1 and b6, and we have generated stable Serpin b1 knockdown KU812-F cell lines by using lentiviral-delivered sh (short hairpin) RNA. The knockdown KU812-F cells (KD cells) produced IL-6 about 2 times higher than KU812-F after administration with toluene diisocyanate (TDI). KD cells also secreted IL-13 upon stimulation with TDI. In contrast, scramble and wild type KU812-F cells did not produce IL-13. we investigated gene alteration between KD cells and scramble KU812-F cells in responses to TDI by mRNA sequencing analysis. We found significant elevation of three genes as follows: CD69 (T cell activation marker), CXCR4 (chemokine receptor), sphingosine-1-phosphate receptor 4 (S1PR4: cell migration). We evaluated the relative fluorescence intensities (RFI) in accordance with human Cell Line Activation Test (H-CRAT). KU812-F and KD cells showed higher RFI of CD69 than scramble cells upon TDI and formaldehyde stimulation, whereas all cell lines did not display any alteration of CD69 expression after DNCB and Methyl Salicilate. In the present LRI research, we investigated the numbers of optimal cells and the expression of CD69 mRNA. We confirmed that KD cells displayed high levels of CD69 expression, and also defined that the numbers of 3×10^4 is adequate for the evaluation. In addition, we found that the levels of CD69 mRNA are significantly augmented in KD cells after TDI stimulation. Collectively, our data suggested that analysis of CD69 expression and IL-13 ELISA is a suitable application for the detection of respiratory sensitizes by using Serpin b1 knockdown KU812-F cells.

Timeline:

2017/3/1-2018/2/28

Topics:

Poster presentation entitled “Development of high sensitivity *in vitro* assessment system of chemical-mediated hypersensitivity by using serine protease inhibitor-deficient cells.” at the annual conference of 2017 LRI, Tokyo, Japan, August 2017

Title of Research:

16_PT04-01

Development of the platform to manage the monitoring data of chemicals in environment, ChemTHEATRE

Principal Investigator:

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Tomohiko Isobe (National Institute for Environmental Studies, Center for Health and Environmental Risk Research)
Nobuaki Ohno (University of Hyogo, Graduate School of Simulation Studies)
Daisuke Ueno (Saga University, Faculty of Agriculture)

Summary of Research:

We have launched a platform to register and visualize the monitoring data of environmental contaminants, named 'ChemTHEATRE' (Chemicals in the THEATRE: Tractable and Heuristic E-Archive for Traceability and Responsible-care Engagement). So far, the data of 58 publications have been registered to the database. Users can find the chemical concentration data in environmental and biological specimens with their metadata, such as sampling date and location, species, biometrics, and so on, in addition to experimental methods including extraction and cleanup processes, used standards, instruments, etc. ChemTHEATRE is now available at http://chem-theatre.com/.

Updated information is provided from Facebook and Twitter.
Facebook: https://www.facebook.com/ChemTHEATRE/
Twitter: https://twitter.com/Chem_THEATRE/

Timeline:

March 1, 2017 – February 28, 2018

Topics:

Our research was presented at ICCA-LRI and JRC Workshop in June 21–22, 2017.
"Introduction to ChemTHEATRE: Open data leads to a new era for integrated exposure and effects analysis."

Publications:

Title of Research:

13_PT05-01-4

Development and practical verification of a comprehensive system to monitor multiple instances of environmental pollution in the Mekong River basin

Principal Investigator:

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Collaborators:

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Summary of Research:

Multiple instances of serious environmental pollution in Southeast Asia have been caused by contamination from agricultural chemicals, endocrine disruptors, heavy metals, and antibiotics. It is therefore important to monitor food and environmental samples from polluted areas to ensure public safety. However, it is difficult to introduce monitoring systems based on expensive, high-quality analytical instruments in areas that often lack other resources. The aim of our project is to develop a novel comprehensive system to monitor contamination in such localities. In this study, we focused mainly on constructing low-cost, easy-to-use, and easy-to-transport paper-based bioassays for antibiotics and endocrine disruptors, in which the biomolecular reactions constructed to detect target chemicals were embedded on paper through the process of freeze-drying. The response signal of this bioassay can be detected by a digital camera and thus does not require expensive conventional laboratory instruments, making it especially suitable in low-resource localities.

Timeline:

November, 2013 -

Topics:

JCIA-LRI 5th Research Report Meeting (Tokyo, August, 2017; Poster presentation)

Publications:

1. 氏家和紀、松浦秀幸、Tran Thi My Duyen、原田和生、平田收正: 「無細胞反応系を用いた抗菌剤検出のための紙基板バイオセンサーの開発」、第 69 回日本生物工学会大会、東京、2017 年 9 月
2. 松浦秀幸、氏家和紀、Tran Thi My Duyen、原田和生、平田收正: 「Development of paper-based biosensors using cell-free system for detection of antibiotics」、2017 年度生命科学系学会合同年次大会、神戸、2017 年 12 月
3. 氏家和紀、松浦秀幸、Tran Thi My Duyen、原田和生、平田收正: 「組換え葉酸合成酵素を用いた紙基板サルファ剤センサーの開発」、日本農芸化学会 関西・中四国・西日本支部 2017 年度合同大阪大会、大阪、2017 年 9 月
4. Kazuki Ujiie, Hideyuki Matsuura, Tran Thi My Duyen, Koki Izutsuu, Kazuo Harada, Hideyuki Matsuura, Kazumasa Hirata: "Paper-based luminescence bioassay method embedding a sequence of enzymatic reactions to detect sulfonamide groups" *submitted*
5. Kazuki Ujiie, Hideyuki Matsuura, Tran Thi My Duyen, Koki Izutsuu, Kazuhito Fujiyama, Shinichiro Maeda, Kazuo Harada, Hideyuki Matsuura, Kazumasa Hirata: "Development of a paper-based luminescence bioassay for therapeutic monitoring of aminoglycosides" *in preparation*

Annual Report 2017

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