LRI

Long-range Research Initiative

Annual Report 2016

2016

Japan Chemical Industry Association

Annual Report 2016

Title of Research:

13_S01-01-3

Development of novel method to evaluate the indicibility of cancer stem cells from iPS cells in chemical compounds

Principal Investigator:

Masaharu Seno, Graduate School of Natural Science and Technology Okayama University

Collaborators:

Tomonari Kasai, Graduate School of Natural Science and Technology Okayama University
Shuichi Furuya, Okayama University Research Administration office
Akifumi Mizutani, Graduate School of Natural Science and Technology Okayama University
Jyunko Masuda, Graduate School of Natural Science and Technology Okayama University
Akimasa Seno, Graduate School of Natural Science and Technology Okayama University

Summary of Research:

Cancer stem cells are typically characterized by continuous proliferation self-renewal as well as by differentiation potential, while stem cells are considered to differentiate into tissue specific phenotype of mature cells under the influence of microenvironment. Cancer stem cells can be traced back to the stem cells under specific influences of microenvironment, so called ‘cancerous niche’, which induces malignant tumors. We have very recently demonstrated the induction of cancer stem cells from mouse iPS cells culturing in the conditioned medium derived from cancer cells, although the details of the mechanisms of differentiation is not very well known as of yet.

In this study, we aim for the development of novel method to evaluate the risk of chemical compounds for the potential to induce cancer stem cells from iPS cells in vitro in a short period. Briefly, mouse iPS cells are suspended in the conditioned medium. The cells are further replenished with the growth medium with the compounds to be assessed.

We are currently observing the fluorescence intensity of GFP, which corresponds to the active Nanog promoter, and establishing the method for detecting the risk of compounds which accrete CSC conversion. The modification for usable method and high sensibility is under way. We are investigating the epigenetic analysis by RRBS (Reduced Representation Bisulfite Sequencing) and DMR (Differentially methylated regions) analysis.

Timeline:

March 2012-February 2016

Publications:

1. Prieto-Vila M, Yan T, Calle AS, Nair N, Hurley L, Kasai T, Kakuta H, Masuda J, Murakami H, Mizutani A, Seno M. iPSC-derived cancer stem cells provide a model of tumor vasculature. Am J Cancer Res. 2016 Sep 1;6(9):1906-1921.
2. Seno A, Kasai T, Ikeda M, Vaidyanath A, Masuda J, Mizutani A, Murakami H, Ishikawa T, Seno M. Characterization of Gene Expression Patterns among Artificially Developed Cancer Stem Cells Using Spherical Self-Organizing Map. Cancer Inform. 2016 Aug 16;15:163-178.
3. Calle AS, Nair N, Oo AK, Prieto-Vila M, Koga M, Khayrani AC, Hussein M, Hurley L, Vaidyanath A, Seno A, Iwasaki Y, Calle M, Kasai T, Seno M. A new PDAC mouse model originated from iPSCs-converted pancreatic cancer stem cells (CSCcm). Am J Cancer Res. 2016 Dec 1;6(12):2799-2815.

Conferences:

1. Takayuki Kudoh, Saki Sasada, Junko Masuda, Masashi Ikeda, Takuma Matsumoto, Anna Sanchez Calle, Neha Nair, Mami Asakura, Tomonari Kasai, Masaharu Seno. Exploration of Target Molecules and Related Pathways Affecting the Conversion of iPSCs into Cancer Stem Cells by Chemical Compounds. IIBMP 2016, (Sep 29-Oct 1,Tokyo)
2. Matumoto T, Sasada S, Ikeda M, Calle AS, Kasai T, Seno M. Elucidation of inducible mechanism of cancer stem cells with chemical compounds. MBSJ 2016, 39 th (Nov 30-Dec 2, Yokohama)

Title of Research:

16_S01-01-2

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

Principal Investigator: Bin-Le Lin, Chief Senior Researcher, Research Institute of Science for Safety and Sustainability, National Institute of Advanced Industrial Science and Technology. 16-1 Onogawa Tsukuba City, Ibaraki, 305-8569 Japan. (Tel) +81-29861 8844, (e-mail) binle-lin@aist.go.jp: Yoshimasa Takahashi, Professor of Toyohashi University of Technology. (Tel) +81-532−446878, (e-mail) taka@cs.tut.ac.jp;

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) Improvement and expansion of hazard DB for PEACH-QSAR system development, and were installed in MeRAM Ver. 2.0; (2) We examined functions and specifications for the data automatic export for PEACH-QSAR system development and start to develop the interface; (3) We examined functions and specifications for connection with KATE and started to develop interfaces; (4) External interface for connection with PEACH-QSAR system was studied, and design and development on detail specifications were carried out; (5) Updated and improved existing functions (effective risk management measures, compliance with the CSCL etc.), and released a new version of MeRAM (Japanese version and English version); (6) We disseminated the tool and Japanese style management of chemicals (CSCL), and hosted an education session of MeRAM at Awajishima ICCA-LRI Workshop, and presented on SETAC meetings. In addition, we accepted student of Toyohashi University of Technology for 2 months (January 9 to February 23, 2017), collaborated on DB analysis of MeRAM and DB creation for PEACH development.

Timeline:

From March 2016 to February 2017

Topics:

None

Publications:

1)(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”.

2) (Invited Speech at Domestic Workshop) Research Committee on Safety Assessment Winter seminar in 2016: "Ecological risk assessment of chemical substances in water environment". December 3, 2016. Jinbocho Mitsui Building, Tokyo.

3) (Paper Publication) Institution magazine "Toxicity Inquiry Box" of the Safety Assessment Committee, 2017: "Ecological risk assessment of chemical substances in water environment".

4) (Presentation at International Meeting) The SETAC North America 37th Annual Meeting, Orlando, Florida, United States. 7-11 November 2016. Oral presentation An all-in-one ecological risk assessment (ERA) tool: AIST-MeRAM.

5) (Research Outcome Exhibition) AIST Techno Bridge Fare, 20-21 October 2016, Tsukuba. IT solution for Cumbersome Ecological Risk Assessment and Management of Chemicals: AIST-MeRAM.

6) (Presentation at International Meeting) The SETAC A/P Conference 2016, Singapore, 14-20 September 2016. A Free Tool (AIST-MeRAM Ver. 2.0) for Multi-purpose Ecological Risk Assessment and Management of Chemicals, Oral presentation.

7) (Presentation at PCD, TISTR, NSTDA) Workshop on Japan-Thailand Bilateral Cooperation on Chemical Management, 3rd-7th October 2016, Bangkok, Thailand.
8) (Invited Speech at Thailand) Risk Assessment of Chemicals. Symposium Capability Building for OECD Guideline for Testing of Chemicals and Ecotoxicology in ASEAN, hosted by Thailand Institute of Scientific and Technological Research (TISTR) & Ministry of Science and Technology (MOST) of Thailand, Chaophya Park Hotel, Bangkok Thailand, 8-9 Aug 2016.

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:

Yoshimasa Takahashi, PhD (Professor, Dept. Comp. Sci. Eng., Toyohashi Univ. Tech.)
1-1 Hibarigaoka, Tempaku-cho, Toyohashi 441-8580 JAPAN
(tel)+81-0532-44-6878, (email)taka@cs.tut.ac.jp

Collaborators:

Tetsuo Katsuragi (Assit. Prof., Toyohashi Univ. Tech.)
Dai Furukawa (Graduate student, Toyohashi Univ. Tech.)
Taku Izumihara (Graduate student, Toyohashi Univ. Tech.)

Summary of Research:

For this year, in order to take a good collaboration with PEACH, which is a desk-top tool for eco-toxicity prediction of chemicals by means of active QSAR modelling, we have constructed a dictionary of chemical structures for the MeRAM because the MeRAM have no chemical structure information excepting chemical name and CAS registry number. The dictionary tool involves the data search functions by CAS number , compound name, molecular formula, full structure search and similar structure search.

We also have investigated the availability of group contribution method based on atomic fragments for fish toxicity prediction of chemical substances. The atomic fragments were originally defined by Viswanadhan et al. in their study on the prediction of molecular properties of logP and molar refraction. To predict the fish toxicity we employed the set of atomic fragments, and determined the toxic fragment constants to the individual atomic fragments with a set of experimental toxicity data of 366 compounds. The toxicity constants gave successful results to the fish toxicity prediction. Alternatively, we investigated estimation of an aqueous solubility parameter (logS) using the atomic fragment method. Those contributions of individual fragments to the logS were determined with the experimental values of 1290 compounds by simplex optimization technique. Five-fold cross validation test gave us the prediction accuracy of the RMSE of 0.73 for the present dataset.

Timeline:

1st Mar. 2016 – 28th Feb. 2017

Topics:

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

Publications:

1) Dai Furukawa, Yuji Ikegami, Tetsuo Katsuragi, Prediction of Fish Toxicity of Chemicals by Atomic Fragment Method: Refinement of hetero atom fragments and optimization of the fragment constants, JSAI2016, Kitakyushu, Jun., 2016.
2) Yoshimasa. Takahashi, Yoshitaka Inagaki, Tetsuo Katsuragi, 21st European Symposium on Quantitative Structure-Activity Relationship (EuroQSAR), Verona, Sep., 2016.
3) Dai Furukawa, Tetsuo Katsuragi, Yoshimasa Takahashi, Fish toxicity prediction of chemicals using atomic fragment method: refinement of the fragments and improvement of the prediction

Title of Research: 16_S01-02

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

Principal Investigator:

Toshihiko Kasahara, PhD (Engineering Manager, FUJIFILM Corporation, Safety Evaluation Center, Ecology & Quality Management Division, CSR Division)
210, Nakanuma, Minamiashigara-shi, Kanagawa 250-0193, Japan
(tel)+81-465-73-7440 (e-mail)ktoshihiko.kasahara@fujifilm.com

Collaborators:

Masaharu Fujita, PhD (FUJIFILM Corporation,Safety Evaluation Center)
210, Nakanuma, Minamiashigara-shi, Kanagawa 250-0193, Japan
(tel)+81-465-73-7440 (e-mail)masaharu.fujita@fujifilm.com
Yusuke Yamamoto (FUJIFILM Corporation,Safety Evaluation Center)
210, Nakanuma, Minamiashigara-shi, Kanagawa 250-0193, Japan
(tel)+81-465-73-7440 (e-mail)yusuke.yamamoto@fujifilm.com
Atsuishi Ono, PhD (Professor, Okayama University, Graduate school of Medicine, Dentistry and Pharmaceutical sciences, Division of Pharmaceutical Sciences)
1-1-1, Tsushimanaka, Kita-ku, Okayama-shi, Okanaya 700-8530, Japan
(tel)086-251-7942 (e-mail) atsushiono3@okayama-u.ac.jp
Hajime Kojima, PhD (Director, National Institute of Health Sciences, Biological safety Research Center, Division of Risk Assessment)
1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
(tel) 03-3700-9874 (e-mail)h-kojima@nihs.go.jp
Shinichi Wantanabe (LION CORPORATION, HUMAN & ENVIRONMENTAL SAFETY EVALUATION CENTER)
100, Tajima, Odawara-shi, Kanagawa 256-0811
(tel)0465-49-4475 (e-mail)shin-w@lion.co.jp
Tsunetsugu Sugawara (LION CORPORATION, HUMAN & ENVIRONMENTAL SAFETY EVALUATION CENTER)
100, Tajima, Odawara-shi, Kanagawa 256-0811
(tel)0465-49-4475 (e-mail) tsune-s@lion.co.jp
Koji Wakabayashi (Manager, MITSUI CHEMICALS, INC., Chemical Safety Department
1144, Togo, Mobara-shi, Chiba 297-0017, Japan
(tel)0475-23-8251 (e-mail)Koji.Wakabayashi@mitsuichemicals.com
Yu Tahara (MITSUI CHEMICALS, INC., Chemical Safety Department
1144, Togo, Mobara-shi, Chiba 297-0017, Japan
(tel)0475-23-8251 (e-mail)Yu.Tahara@mitsuichemicals.com
Nobuyuki Horie (Senior Research Scientist, SUMITOMO Chemical Co., Ltd., Environmental Health Science Laboratory)
3-1-98, Kasugadenaka, Konohana-ku, Osaka-shi, Osaka 554-8558, Japan
(tel)06-6466-5107 (e-mail)horien@sc.sumitomo-chem.co.jp
Keiichi Fujimoto (SUMITOMO CHEMICAL Co., Ltd., Environmental Health Science Laboratory)
3-1-98, Kasugadenaka, Konohana-ku, Osaka-shi, Osaka 554-8558, Japan
(tel)06-6439-9753 (e-mail) fujimotok2@sc.sumitomo-chem.co.jp
Kei Kusakari (NISSAN CHEMICAL INDUSTRIES, LTD. Biological Research Laboratories)
1470, Shiraoka, Shiraoka-shi, Saitama 349-0294, Japan
(tel)0480-92-2513 (e-mail)kusakarik@nissanchem.co.jp
Yoshihiko Kurokawa (NISSAN CHEMICAL INDUSTRIES, LTD. Biological Research Laboratories)
1470, Shiraoka, Shiraoka-shi, Saitama 349-0294, Japan
(tel)0480-92-2513 (e-mail)kurokawayo@nissanchem.co.jp

Takashi Sozu, PhD (Assistant Professor, Department of Information and Computer Technology, Faculty of Engineering, Tokyo University of Science)
6-3-1, Shinjuku, Katsushika-ku, Tokyo 125-8585, Japan
(tel)03-5876-1688 (e-mail)sozu@rs.tus.ac.jp
Takuto Nakayama (Department of Management Science, Graduate School of Engineering, Tokyo University of Science)
6-3-1, Shinjuku, Katsushika-ku, Tokyo 125-8585, Japan
(tel)03-5876-1688 (e-mail)4416624@ed.tus.ac.jp
Takeru Kusao (Department of Management Science, Faculty of Engineering, Tokyo University of Science)
6-3-1, Shinjuku, Katsushika-ku, Tokyo 125-8585, Japan
(tel)03-5876-1688 (e-mail)j4413022@ed.tus.ac.jp
Tsuyoshi Kawakami, PhD (Director, National Institute of Health Sciences, Biological safety Research Center, Division of Risk Assessment)
1-18-1, Kamiyoga, Setagaya-ku, Tokyo 158-8501, Japan
(tel) 03-3700-1141 (e-mail)tkawa@nihs.go.jp
Kohichi Kojima、PhD （Director-General, Food and Drug Safety Center, Hatano Research Institute）
729-5, Ochiai, Hadano-shi, Kanagawa 257-8523, Japan
(tel)0463-82-4751 (e-mail)kojima.k@fdsc.or.jp
Nicol C. Kleinstreuer, PhD （NTP Interagency Center for the Evaluation of Alternative Toxicological methods）
(tel) (919) 541-7997 (e-mail) nicole.kleinstreuer@nih.gov
Jon Richmind, PhD （Ethical Biomedical Research and Testing Advice & Consultancy）
Doubledykes Carslogie Road, Cupar, Fife KY154HY, United Kingdom
(tel)077-86-381-757 (e-mail)dr.richmond@gmail.com
Bae-Hwan Kim, PhD （Professor, Keimyung University, Department of Public Health, College of Natural Science）
1095, Dalgubeol-daero, Dalseo-Gu, Daegu 42601, Republic of Korea
(tel)+82-53-580-5933 (e-mail)kim9399@kmu.ac.kr

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.

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.

The Pre-training test was performed using 5 test chemicals to be easy to be categorized correctly. The predictive results for 5 chemicals at four participating laboratories were completely consistent with the results at lead laboratory, so it was thought that the first step of technical transfer was finished. The Training test was performed using 10 test chemicals that would be difficult to be categorized correctly. The Within-Laboratory Reproducibility at each laboratory were 100%, 90%, 100% and 100%, respectively, and so it was thought that technical transfer was completely finished because the goal was accomplished.

It was decided at VMT meeting that Phase-1 study (as Within-Laboratory Reproducibility) would be performed using three replicate sets of 10 chemicals and Phase-2 (as Between-Laboratory Reproducibility) study would be performed using one set of 30 chemicals in the ADRA validation study. The Within-Laboratory Reproducibility were 100% (10/10) , 100%(10/10), 100%(7/7) and 90%(9/10) and these values were higher than expected in that the results of Phase-1 achieved the Within-Laboratory Reproducibility of more than 80% of target.

After Phase-1 validation study, VMT teleconference was held on January in 2019, and VMT member were evaluated the results of Phase-1 study. Because all the improvement items proposed by lead laboratory were approved by VMT members, and the Phase-2 study was started on February in 2019 as scheduled.

Timeline:March 1, 2016–

Topics:

1. Presented at ICCA-LRI & NIHS WORKSHOP-Awaji Island, Japan「Validation study on Amino acid Derivative Reactivity Assay (ADRA) for prediction of skin sensitization」

Publications:

1. Presented at the 29th Annual Meeting of the Japanese Society of Alternatives to Animal Experiments (November 18, 2016) 「Reporting of Training and Transfer Outcome for Validation Study of Alternative Method (ADRA) for Skin Sensitization using Cys and Lys Derivatives」
2. Planed to be presented at the 44th Annual Meeting of the Japanese Society of Toxicology (July 10-12, 2017) 「Multicenter Validation Study of Novel in chemico Skin Sensitization Assay (ADRA): 1st Report」

Title of Research:

13_S03-01-3

Study on pathogenic mechanism of sick building syndrome by utilizind Drosophila and development of evaluation system of causative materials

Principal Investigator:

Kaeko Kamei, PhD (Professor, Kyoto Institute of Technology, Department of Biomolecular Engineering)
Matsugasaki, Sakyo-ku, Kyoto, 606-8585, Japan
(tel) +81-75-724-7553, (e-mail) kame@kit.ac.jp

Collaborators:

Summary of Research:

This study aims to elucidate the mechanism of onset of sick building syndrome using *Drosophila* and to develop a risk assessment method of causative substances. Based on the results of previous studies, we have reported that the exposure to formaldehyde gas and *o*-xylene gas causes the change in the concentrations of several proteins in Drosophila and that and that similar change are induced by aging. These results suggest that biological reactions due to gas exposure may be related to aging.

In this study, we have revealed that the exposure to formaldehyde gas or o-xylene gas shortens the life span of *Drosophila*. The expression of aging marker molecules increased in Drosophila by 20-days exposure. Analysis of insulin signal also showed the possibility that anti-aging system was suppressed by exposure to gas.

We analyzed the effects of formaldehyde gas exposure or *o*-xylene gas exposure on the oxidative stress, since oxidative stress is considered to be the cause of accelerated aging. The results indicated that the oxidative stress might be increased in the initial stage of exposure. However, the long-term exposure led to no significant difference in oxidative stress from non-exposed group. The reason for this is unknown, but some adaptation by *Drosophila* is considered. It is necessary to analyze more details.

We have reported that expression of actin decreases due to formaldehyde gas exposure. In this study, we examined the *Drosophila* carrying a GFP gene downstream from the promoter of actin 88F as a formaldehyde evaluation system. We obtained the results suggesting that the recombinant *Drosophila* used is useful as a simple formaldehyde evaluation system by the observation of the pupa under the fluorescence microscope.

Timeline:

March 1, 2016 – February 28, 2017

Topics:

Drosophila is well known as a model for human disease. In this study, we found that the life time of *Drosophila* was shorten by exposing formaldehyde gas or *o*-xylene gas. We showed that the gas exposure may suppress the anti-aging system in *Drosophila*.

Publications:

Research on the effects of chemical substances on children, elderly people, and those with gene disorders

Title of Research:

15_S03-01 (PS)

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

Principal Investigator:

Takayuki Yoshimoto, PhD (Professor, Tokyo Medical University, Institute of Medical Science)
6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan
(tel)+81-3-3351-6141 Ext. 431, (e-mail) yoshimot@tokyo-med.ac.jp

Collaborators:

Izuru Mizoguchi, PhD (Research Associate, Tokyo Medical University, Institute of Medical Science) 6-1-1 Shinjuku, Shinjuku-ku, Tokyo 160-8402, Japan
(tel) +81-3-3351-6141 Ext. 448, (e-mail) imizo@tokyo-med.ac.jp

Summary of Research:

Recently, the use of animal models in safety testing of chemicals will be significantly limited due to introduction of 3Rs principles of animal experimentations in research wherever possible. Therefore, several in vitro assays to predict the respiratory sensitizing potential of chemicals have been developed. 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 respiratory sensitizers from chemical 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.

First of all, we established an immunocompetent 3D co-culture system of human upper airway using the Alvetex® scaffold from Reinnervate Ltd. It consists of a human airway epithelial cell line BEAS-2B, immature dendritic cells (DCs) derived from peripheral blood $CD14^+$ monocytes, and a fibroblast cell line MRC-5 cultured in individual scaffolds, and these scaffolds were then assembled into a 3D multi-cell tissue model, which more closely mimics the in vivo situation. Then, three typical chemicals for respiratory sensitizers such as phthaldialdehyde, hexamethylene diisocyanate, and trimellitic anhydride and skin sensitizers such as oxaloacetic acid, formaldehyde, and dinitrochlorobenzene disolved in DMSO diluted with medium were added on the 3D co-culture system. After incubation for 9 hours, total RNA was extracted and subjected to real-time RT-PCR to analyze the expression levels of 14 molecules, which are critical for induction of Th2 differentiation and Th2 immune responses such as OX40L, CCR7, IL-10, IL-33, and thymic stromal lymphopoietin. These sensitizers showed similarly augmented expression of DC maturation markers such as CD86, but among these molecules OX40L expression in DCs was most greatly enhanced by the respiratory sensitizers as compared with that by the skin sensitizers.

The present results indicate that this 3D co-culture system has great potential to contribute to the establishment of in vitro evaluation system for the prediction of respiratory sensitizing potential of chemicals by means of enhanced expression of key molecules such as OX40L as markers in DCs.

Timeline:

March 1, 2016-Feberary 28, 2017

Topics:

ICCA-LRI and NIHS Workshop, poster presentation “Establishment of a novel in vitro evaluation system for the prediction of respiratory sensitizing potential of chemicals” (Awaji Island, June 15-16, 2016)

Publications:

The 45th Annual Meeting of The Japanese Society for Immunology, poster presentation “Development of a novel 3D co-culture system for evaluation of respiratory sensitizing potential” (Okinawa, December 5-7, 2016)

Title of Research:

13_PT01-01

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

Principal Investigator:

Yuhei Nishimura (Department of Pharmacogenomics, Mie University Graduate School of Medicine)

Collaborators:

Toshio Tanaka and Reiko Kawase (Department of Systems Pharmacology, Mie University Graduate School of Medicine)

Summary of Research:

Various chemicals may affect the differentiation of neurons, oligodendrocytes and astrocytes, which may cause neurodevelopmental disorders such as autism and attention deficit hyperactive disorder. *In vivo* models that can be used in both visualization of the neuronal differentiation and quantitative assessment of behavior are highly valuable for developmental neurotoxicity testing. 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 three-color zebrafish exhibiting blue, yellow and red fluorescence in neurons, oligodendrocytes and astrocytes, respectively. Using the zebrafish, we evaluated the developmental neurotoxicity of four positive control chemicals, valproic acid, methyl mercury, nicotine, and bisphenol A. We were able to detect the toxicity of these chemicals on the neuronal differentiation. We will use more positive and negative chemicals to examine the sensitivity and specificity of the *in vivo* fluorescent imaging of neuronal differentiation in zebrafish for developmental neurotoxicity testing.

Timeline:

Mar 2016 ~ Feb 2017

Publications:

1) **Activation of Sterol Regulatory Element Binding Factors by Fenofibrate and Gemfibrozil Stimulates Myelination in Zebrafish**. Ashikawa, Y., Nishimura, Y., Okabe, S., Sasagawa, S., Murakami, S., Yuge, M., Kawaguchi, K., Kawase, R., and Tanaka, T. *Frontiers in pharmacology* 7, 206 (2016)
2) **EP300 protects from light-induced retinopathy in zebrafish.** Kawase, R., Nishimura, Y., Ashikawa, Y., Sasagawa, S., Murakami, S., Yuge, M., Okabe, S., Kawaguchi, K., Yamamoto, H., Moriyuki, K., Yamane, S., Tsuruma, K., Shimazawa, M., Hara, H., and Tanaka, T. *Frontiers in pharmacology* 7, 126 (2016).
3) **E2F4 promotes neuronal regeneration and functional recovery after spinal cord injury in zebrafish.** Sasagawa, S., Nishimura, Y., Hayakawa, Y., Murakami, S., Ashikawa, Y., Yuge, M., Okabe, S., Kawaguchi, K., Kawase, R., and Tanaka, T. *Frontiers in pharmacology* 7, 119 (2016).
4) **Downregulation of GSTK1 Is a Common Mechanism Underlying Hypertrophic Cardiomyopathy.** Sasagawa, S., Nishimura, Y., Okabe, S., Murakami, S., Ashikawa, Y., Yuge, M., Kawaguchi, K., Kawase, R., Okamoto, R., Ito, M., and Tanaka, T. *Frontiers in pharmacology* 7, 162 (2016).
5) **Comparative transcriptome analysis identifies CCDC80 as a novel gene associated with pulmonary arterial hypertension.** Sasagawa, S., Nishimura, Y., Sawada, H., Zhang, E., Murakami, S., Ashikawa, Y., Yuge, M., Okabe, S., Kawaguchi, K., Kawase, R., Mitani, Y., Maruyama, K., and Tanaka, T. *Frontiers in pharmacology* **7**, 142 (2016).
6) **Integrated Approaches to Drug Discovery for Oxidative Stress-Related Retinal Diseases.** Nishimura, Y., and Hara, H. *Oxid Med Cell Longev.* 2370252 (2016).

Title of Research:

13_PT01-03-2

Development of a combined in vitro/in silico system to predict and evaluate the complex hepatotoxicity of chemical compounds.

Principal Investigator:

Kouichi Yoshinari, Ph.D. (Professor, University of Shizuoka, School of Pharmaceutical Sciences)
52-1 Yada, Suruga-ku, Shizuoka 422-8526
(Tel/fax) 054-264-5685 (e-mail)yoshinari@u-shizuoka-ken.ac.jp

Collaborators:

Hiroyuki Kojima (Hokkaido Institute of Public Health)

Summary of Research:

The aim of study is to develop a system for toxicity evaluation and prediction. In this study. we measured the in vitro reactivity to xenobiotic-responsive nuclear receptors and cytochrome P450s of the chemicals in HESS toxicity database, which contains rat repeated dose toxicity test data of Chemical Substances Control Law-related substances and is available from National Institute of Technology and Evaluation. The results indicate that those biological assay data for nuclear receptors and cytochrome P450s can be used as different types of molecular descriptors from those calculated in silico based on chemical structures. Then, the in vitro assay data and in silico molecular descriptors were subjected separately or in combination to hierarchical clustering and the resulting groupings were compared. The results suggest that the addition of in vitro assay data is useful for grouping chemicals showing similar toxicity properties. Although verification with more large numbers of chemicals is needed, our findings will help to establish a novel toxicity-predicting system based on grouping/categorization and read-across methods.

Timeline:

March 2016 – February 2017

Topics:

None

Publications:

Journal article

1. A Masuda, K Yoshinari, et al.: Construction of the database of rat repeated-dose toxicity tests for the toxicological characterization of hepatocyte hypertrophy. *Yakugaku Zasshi*, 2017 in press.

Oral/poster presentation

1. K Yoshinari, H Oki, T Sasaki, T Hosaka, J Takeshita: New approach for the prediction of repeated-dose toxicity using in vitro and in silico methods in combination with in vivo toxicity database. ICCA-LRI and NIHS Workshop. Jun. 2016, Awaji, Japan.
2. K Yoshinari, H Nakayama, et al.: Attempt on the in silico prediction of rat hepatotoxicity using hierarchical clustering with chemical descriptors. 29th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments. Nov. 2016, Fukuoka.
3. M Watanabe, K Yoshinari, et al.: Biological profiling of chemical compounds based on the inhibition of cytochrome P450 activities. 29th Annual Meeting of the Japanese Society for Alternatives to Animal Experiments. Nov. 2016, Fukuoka.
4. T Sasaki, K Yoshinari, et al.: Inhibitory effects on human cytochrome P450s of chemical compounds and their associations with hepatotoxicity. 137th Annual Meeting of the pharmaceutical Society of Japan. Mar. 2017, Sendai.
5. Y Kitsunai, K Yoshinari, et al.: Search for novel functions of PXR and CAR using in vivo toxicity data. 137th Annual Meeting of the pharmaceutical Society of Japan. Mar. 2017, Sendai.

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:

Objective: 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) with c-Myc tag 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*. We here established a new *in vivo* screening assay using E-Rep mice with *in vivo* bioluminescence imaging to detect the estrogenic effects of xenobiotics and validated the assay to compare with 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) for 7 consecutive days. The Luc activity was monitored in living animals with IVIS imaging at Day 1, 2, 4, 7. After last detection of *in vivo* bioluminescence imaging, mice were dissected and then the estrogenic effects were evaluated by measurement of uterine weight or detection of Luc activity at Day 7.

Results: Luc activity in the uterus increased significantly when EE was administered at 3 μg/kg/day or greater at Day 7. In contrast, the uterine weight rose significantly only when estradiol was administered at 10 μg/kg/day. In addition, *in vivo* bioluminescence imaging analysis could detect significant increment of Luc activity at above 0.3 μg/kg/day of EE, suggesting that detection of Luc activity in E-Rep mice by *in vivo* bioluminescence imaging provide a more sensitive assay of estrogenic activity than the uterotrophic bioassay.

Conclusion: E-Rep mice with *in vivo* bioluminescence imaging provide a potential new assay system to measure estrogenic activity of endocrine disrupting chemicals in multiple organs with high sensitivity.

Timeline: April, 2016 – March, 2017

Topics:

1) Our research paper “Fluorene-9-bisphenol is anti-oestrogenic and may cause adverse pregnancy outcomes in mice” published in *Nature Communications* was highlighted in natureasia.com (http://www.natureasia.com/en/research/highlight/11678).
2) Our research paper “Fluorene-9-bisphenol is anti-oestrogenic and may cause adverse pregnancy outcomes in mice” published in *Nature Communications* featured as a daily news

entitled “BPA-free water bottles may contain another harmful chemical” in *New Scientist* (https://www.newscientist.com/article/2123098-bpa-free-water-bottles-may-contain-another-harmful-chemical/).

Publications:

1) Zhang Z, Hu Y, Guo J, Yu T, Sun L, Xiao X, Zhu D, Nakanishi T, Hiromori Y, Li J, Fan X, Wan Y, Cheng S, Li J, Guo X, Hu J, Fluorene-9-bisphenol is anti-oestrogenic and may cause adverse pregnancy outcomes in mice, *Nature Communications* 8:14585 (2017)

Title of Research:

16_PT01-01

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

Principal Investigator:

Shunichi Takeda, MD, PhD (Professor, Kyoto University, Graduate School of Medicine, Department of Radiation Genetics)
Yoshida-Konoecho, Sakyo-ku, Kyoto 606-8501, Japan
(tel)+81-(0)75-753-4410, (email) stakeda@rg.med.kyoto-u.ac.jp

Collaborators:

Masamitsu Honma (National Institute of Health Sciences, Division of Genetics and Mutagenesis)
1-18-1, Kamiyouga, Setagaya, Tokyo 158-8501, Japan
(tel)+81-(0)3-3700-1141, (email) honma@nihs.go.jp

Manabu Yasui (National Institute of Health Sciences, Division of Genetics and Mutagenesis)
1-18-1, Kamiyouga, Setagaya, Tokyo 158-8501, Japan
(tel) +81-(0)3-3700-1141, (email) m-yasui@nihs.go.jp

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) Generation of *XPA*$^{-/-}$*/XRCC1*$^{-/-}$ TK6 cells

We created *XPA*$^{-/-}$*/XRCC1*$^{-/-}$ TK6 cells.

Timeline:

March 1, 2016-

Topics:

ICCA-LRI and NIHS Workshop, “Establishment of method of characterizing DNA lesions caused by industrial chemical compounds”

Publications:

Masataka Tsuda, Kazuhiro Terada, Masato Ooka, Koji Kobayashi, Hiroyuki Sasanuma, Ryo Fujisawa, Toshiki Tsurimoto, Junpei Yamamoto, Shigenori Iwai, Kei Kadoda, Remi Akagawa, Shar-yin Naomi Huang, Yves Pommier, Julian E. Sale, Shunichi Takeda, and Kouji Hirota.
“The Dominant Role of Proofreading Exonuclease Activity of Replicative Polymerase □ in Cellular Tolerance to Cytarabine (Ara-C).” *Oncotarget (in press)*
(The first author belongs to Takeda lab.)

Title of Research:

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

Principal Investigator:

Yoshihiro Uesawa

Summary of Research:

QSAR models for activities of Adverse Outcome Pathways (AOPs) were constructed using machine learning methods. Moreover, a chemical structure-AOP-adverse effect database was constructed from the Tox21-AOP database and other adverse effect databases, such as JADER in Japan and FEARS in the USA. As a result of analyses of these integrated databases, random forest models indicated good prediction performance. Furthermore, it was showed that many kinds of adverse effects are related to AOP activities in the database.

Timeline:

March, 2016 – February, 2017

Topics:

Poster presentation, “Prediction for Activators of Adverse Outcome Pathways based on the Tox21 10K Compound Library”, ICCA-LRI and NIHS Workshop

Publications:

1. Yoshihiro Uesawa, "High-performance prediction of adverse outcome pathways based on machine learning methods with chemical structures", 31st JSSX Annual Meeting, symposium, invited lecture (invited), 2016/10/15
2. Yoshihiro Uesawa, "QSAR prediction of adverse outcome pathways based on machine learning methods", In-House Lecture, Sumitomo Chemical Company, Limited (invited), 2016/9/26
3. Yoshihiro Uesawa, "Construction of QSTR prediction models based on chemical structures"CBI Annual Meeting 2016, Focused session (invited), 2016/10/25
4. Yoshihiro Uesawa, "Analyses of chemical structures related to adverse outcomes based on large scale database", CBI Annual Meeting 2016, Focused session (invited), 2016/10/27
5. Yoshihiro Uesawa, "The Development of Highly Accurate Toxicity Prediction Methods Using the JMP® Machine Learning Function", Discovery Summit Japan, 2016/11/18
6. Jun Teraoka, Yurie Yoshida, Ryota Higuchi , Yuhei Mashiyama, Yoshihiro Uesawa, "Study of Effects of NF-κB for Reporting Frequency of Adverse Drug Reactions based on Tox21-AOP Database", CBI Annual Meeting 2016, 2016/11/27
7. Yuhei Mashiyama, Yurie Yoshida, Ryota Higuchi , Jun Teraoka, Yoshihiro Uesawa, "Analyses of Chemical Properties Associated with Reporting Frequency of Erythema Exudativum Multiforme Major in Adverse Drug Reaction Database", CBI Annual Meeting 2016, 2016/11/27
8. Ryota Higuchi, Yurie Yoshida, Jun Teraoka, Yuhei Mashiyama, Yoshihiro Uesawa, "Analyses of Chemical Properties Associated with Reporting Frequency of Interstitial Lung Disease in Adverse Drug Reaction Database", CBI Annual Meeting 2016, 2016/11/27
9. Yurie Yoshida, Ryota Higuchi, Jun Teraoka, Yuhei Mashiyama, Yoshihiro Uesawa, "Analyses of Chemical Properties for Reporting Frequency of Thrombocytopenia in Adverse Drug Reaction Database", CBI Annual Meeting 2016, 2016/11/27
10. Yoshihiro Uesawa, "Dragon descriptors develop computational toxicology"The seminar on the Dragon descriptors (invited), 2016/12/22

Title of Research:

15_PT02-01

Construction of novel *in vitro* evaluation systems based on genotoxic mechanisms of nanomaterials

Principal Investigator: **Yukari Totsuka, Ph.D., Natl. Cancer Centr. Res. Inst.**

Collaborators:

Dai Nakae, M.D., Ph.D. (Professor, Laboratory of Food Safety Assessment Science, Department of Nutritional Science and Food Safety, Faculty of Applied Biosciences, Tokyo University of Agriculture),

Masatoshi Watanabe, M.D., Ph.D. (Professor, Yokohama National University, Graduate School of Engineering),

Koichiro Hayashi (Division on Materials Research, Institute of Materials and Systems for Sustainability, Nagoya University)

Summary of Research: ***To establish new in vitro evaluation systems for lung/skin toxicity of nanomaterials, we aimed to establish assay models as listed below.***

① A novel in vitro genotoxicity assay model to assess lung toxicity using a co-culture system.

② A novel in vitro assay model to assess skin toxicity using 3D human skin reconstitution models.

③ A novel in vitro assay model using 3D culture techniques such as tissue-slice and spheroids.

For the lung toxicity test, we established a co-culture system of GDL1 and RAW264 cells. We evaluated nanomaterials having differences in physicochemical character by using this co-culture system. In the present study, we chose multi-walled carbon nanotubes (MWCNTs) with different sizes, and magnetite (MGT) with/without surface modification as test nanomaterials. When gpt delta mice were intratracheally instilled with multiple doses of different sizes of MWCNTs, mutation frequency (MF) observed in the lungs was higher than that of the vehicle control. However, the influence of size differences against MF was not observed in the lungs of mice exposed to these MWCNTs. Supporting this, a similar result was observed in the co-culture assay system. We also evaluated MF induced by MGT with/without surface modification using the co-culture system, and there was a tendency that higher MF was observed in the cells exposed to MGT with surface modification than that of MGT without surface modification. Therefore, it is suggested that the co-culture

assay model can be considered as a suitable evaluation system for nanomaterial toxicity for the lungs.

As a 3D skin model, we selected the LabCyte EPI model. Using biochemical and histopathological techniques, we assessed cytotoxicity and ability of gold and silver nanoparticles to invade into the skin using a reconstituted 3D human skin model (LabCyte EPI model, Japan Tissue Engineering Co., Ltd.) and HepG2 cells. In the reconstituted 3D human skin model, neither gold nor silver nanoparticles killed or invaded into the epidermis up to 1000 μg/mL, while neither nanoparticle killed HepG2 cells up to 100 μg/mL. It is thus suggested that gold or silver nanoparticles do not possess cytotoxicity or ability to invade into the skin, at least under the present experimental conditions. Furthermore, the LabCyte EPI model may be useful as a novel in vitro system to assess percutaneous toxicity of nanomaterials.

On the other hand, since the respiratory system is susceptible to damage resulting from inhalation of nanomaterials, the assay of A549 spheroids layered on histological sections was used for MGT induced cytotoxicity. Results of spheroid disruption by cellular adhesion damage and cell viability by Alamar Blue assay showed the possibility of the assay of A549 spheroids layered on histological sections as a toxicological safety test.

Timeline: March 1st, 2016 – February 29th, 2017

Topics: “Construction of novel in vitro evaluation systems based on genotoxic mechanisms of nanomaterials “ Presented at the poster session of the Annual Conference of New JCIA-LRI

Publications:

Journals:

1. KatoT, Toyooka T, Ibuki Y, Masuda S, Watanabe M, Totsuka Y. Effect of Physicochemical Character Differences on the Genotoxic Potency of Kaolin. Genes Environ., in press.
2. Koichiro Hayashi, Yoshitaka Sato, Wataru Sakamoto, Toshinobu Yogo, “Theranostic Nanoparticles for MRI-Guided Thermochemotherapy: Tight Clustering of Magnetic Nanoparticles Boosts Relaxivity and Heat-Generation Power” ACS Biomaterials Science & Engineering, 3, 95–105, 2017.

Meetings:

1. TotsukaY, Watanabe M, Hayashi K, Nakae D: Development of a novel in vitro mechanism-based evaluation system of the genotoxicity of nanomaterials 45th EEMGS(Copenhagen、Aug, 2016)
2. Sato H, Sakamoto Y, Nakae D, Totsuka Y: Effect of Physicochemical Character Differences on genotoxic potency of MWCNTs 45th JEMS (Tsukuba、Nov, 2016)

Research on the effects of chemical substances on children, elderly people,and those with gene disorders

Title of Research:

12_PT03-01-4

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:

Akira Nakamura, Department of Immunology, Tohoku Medical and Pharmaceutical University
4-4-1 Komatshushima, Aoba-ku, Sendai, Japan 981-8558 (tel) +81-22-727-0212
(e-mail) aki-n@tohoku-mpu.ac.jp

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. In the present LRI research, 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). Because commercially available SIPR4 antibodies recognize the intracellular domain, we examined the expression of CD69 and CXCR4 by flow cytometric analysis upon stimulation with TDI and formaldehyde, a skin sensitizer Dinitrochlorobenzene (DNCB), and control Methyl Salicilate. We evaluated the relative fluorescence intensities (RFI) in accordance with human Cell Line Activation Test. 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. On the other hand, we could not detect CXCR4 expression in these cell lines. Taken together, 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:

2016/3/1-2017/2/28

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 ICCA-LRI and NIHS Workshop, Awaji Island, Japan, June, 2016

Research on the effects of chemical substances on children, elderly people,and those with gene disorders

Title of Research:

13_PT03-01-3

Quantitative prediction of the pharmacokinetics of chemical substances by the use of mathematical model with considering the age-dependent functional changes of metabolic enzymes and transporters

Principal Investigator:

Hiroyuki Kusuhara, PhD (Professor, Lab. of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo)
7-3-1 Hongo, Bunkyo-ku Tokyo 113-0033 JAPAN
(tel)+81-3-5841-4770, (e-mail) kusuhara@mol.f.u-tokyo.ac.jp

Collaborators:

Kazuya Maeda, PhD (Associate Professor, Lab. of Molecular Pharmacokinetics, Graduate School of Pharmaceutical Sciences, The University of Tokyo)
7-3-1 Hongo, Bunkyo-ku Tokyo 113-0033 JAPAN
(tel)+81-3-5841-4772, (e-mail) kmaeda@mol.f.u-tokyo.ac.jp

Yuichi Sugiyama, PhD (Head, Sugiyama Laboratory, RIKEN Innovation Center, RIKEN Cluster for Industry Partnerships, RIKEN)
1-6 Suehiro-cho, Tsurumi-ku, Yokohama, Kanagawa 230-0045 JAPAN
(tel)+81-45-503-9211, (e-mail) ychi.sugiyama@riken.jp

Ichiro Ieiri, PhD (Professor, Dept. of Clinical Pharmacokinetics, Graduate School of Pharmaceutical Sciences, Kyushu University)
3-1-1 Maidashi, Higashi-ku Fukuoka 812-8582 JAPAN
(tel)+81-92-642-6656, (e-mail) ieiri-ttr@umin.ac.jp

Summary of Research:

To predict the influence of age on the pharmacokinetics of chemical substances, we performed a human clinical cocktail semi-microdosing study for investigating the age-dependent functional changes of metabolism and transport of compounds with healthy adults and aged persons. As a result, pharmacokinetics of some probe drugs for CYP (cytochrome P450) 3A and OATP (organic anion transporting polypeptide) transporters were changed in aged persons. This suggests that functions of intestinal and hepatic CYP3A/OATPs might be altered in aged persons. Moreover, we measured the plasma and urine concentration of trimethylamine N-oxide (TMAO) to investigate whether this can be used as a renal OCT2 functional probe endogenous compound. Although TMAO is taken up into kidney mainly via OCT2 in mice, since the contribution of renal secretion mediated by OCT2 to the overall renal clearance is fairly minor in humans, TMAO cannot be used as a sensitive probe for renal OCT2 in humans.

Timeline:

March 1, 2016 -

Topics:

2016 ICCA-LRI Workshop (poster) “Quantitative prediction of the pharmacokinetics of chemical substances by the use of mathematical model with considering the age-dependent functional changes of metabolic enzymes and transporters”

Publications:

Takeshi Miyake, Tadahaya Mizuno, Hiroyuki Kusuhara “Organic cation transporter (OCT) is responsible for the renal influx and clearance of trimethylamine N-oxide (TMAO)”, 31st Japan Society of the Study of Xenobiotics (JSSX) Annual meeting, Nagano, 2016.10

Title of Research:

16_PT04-01

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

Principal Investigator:

Kei Nakayama, PhD (Assistant Professor, Ehime University, Center for Marine Environmental Studies)
2-5 Bunkyo-cho, Matsuyama 790-8577, Japan
(tel) +81-89-927-8132, (e-mail) kei_n@ehime-u.ac.jp

Collaborators:

Tatsuya Kunisue (Ehime University, Center for Marine Environmental Studies)
Itsuki C Handoh (Niigata University, Educational Strategy Coordination Office)
Seiichi Uno (Kagoshima University, Faculty of Fisheries)
Tomohiko Isobe (National Institute for Environmental Studies, Center for Health and Environmental Risk Research)
Nobuaki Ohno (University of Hyogo, Graduate School of Simulation Studies)

Summary of Research:

We are now developing 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 27 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, 2016 – February 28, 2017

Topics:

Our research was presented at ICCA-LRI and NIHS Workshop in June 15–16, 2016.
"Development of the platform to manage the monitoring data of chemicals in environment, ChemTHEATRE."

Publications:

Title of Research:

13_PT05-01-3

Development and practical verification of a novel comprehensive monitoring system for multiple contaminations of environmental pollutants in the Mekong River basin

Principal Investigator:

Kazumasa Hirata, PhD (Professor, Osaka University, Pharmaceutical Sciences; 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan)
(tel) +81-06-6879-8236, (e-mail) hirata@phs.osaka-u.ac.jp

Collaborators:

Kazuo Harada, PhD (Osaka University, Pharmaceutical Sciences); Hideyuki Matsuura, PhD (Osaka University, Pharmaceutical Sciences); Yoshinori Sumimura (Osaka University, Global Collaboration Center); Nguyen Quang Trung (Institute of Environmental Technology, Department of Environmental Toxic Analysis); Misa Muraoka (Osaka University, Pharmaceutical Sciences); Tran Thi My Duyen (Can Tho University, College of Aquaculture and Fisheries); Jun-ichi Nishikawa, PhD (Mukogawa Women's University, School of Pharmacy and Pharmaceutical Sciences); Taku Yamashita (Mukogawa Women's University, School of Pharmacy and Pharmaceutical Sciences)

Summary of Research:

Serious environmental pollution of the international river basins of Southeast Asia, such as the Mekong River basin, has been caused by multiple contaminations of pollutants such as agricultural chemicals, endocrine disruptors, heavy metals, and antibiotics. It is, therefore, important to monitor food and environmental samples from these areas to ensure public safety. The aim of our project is to develop a novel comprehensive monitoring system for multiple contaminations, for use in ASEAN countries. In the present study, we mainly focused on constructing biosensors for sulfonamides that inhibit bacterial folate synthesis and other antibiotics that inhibit bacterial protein synthesis. Each antibiotic was detected using a series of enzymatic reactions performed on pattern-printed papers, such as those including a folate synthetic enzyme or in vitro protein synthesis, in a low-cost, easy-to-use, and easy-to-transport manner. The response signal of the paper-based biosensor can be detected by the naked eye or a digital camera, and, therefore, does not require high-cost conventional laboratory instruments, making it especially suitable in low-resource localities.

Timeline:

November, 2013 -

Topics:

2016 ICCA-LRI and NIHS workshop "Development of novel biosensors for detecting environmental pollutants"

Publications:

1. Tran Thi My Duyen, Hideyuki Matsuura, Kazuki Ujiie, Misa Muraoka, Kazuo Harada, Kazumasa Hirata: "Paper-based colorimetric biosensor for antibiotics inhibiting bacterial protein synthesis", *Journal of Bioscience and Bioengineering*, 123(1): 96-100, 2017
2. 氏家和紀、Tran Thi My Duyen、松浦秀幸、村岡未彩、原田和生、平田收正: 「タンパク質合成阻害作用を持つ抗菌性物質検出のための紙を基盤とする比色センサーの開発」、第 65 回日本分析化学会年会、札幌、2016 年 9 月
3. Tran Thi My Duyen, Hideyuki Matsuura, Kazuki Ujiie, Misa Muraoka, Kazuo Harada, Kazumasa Hirata: "Paper-based colorimetric biosensor for antibiotics targeting bacterial protein synthesis", 第 68 回日本生物工学会大会、富山、2016 年 9 月
4. Tran Thi My Duyen, Kazuki Ujiie, Misa Muraoka, Kazuo Harada, Hideyuki Matsuura, Kazumasa Hirata: "Development of paper-based biosensor for detection of antibiotics targeting protein synthesis", Biosensors 2016, Gothenburg, Sweden, May 2016

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Japan Chemical Industry Association

1-4-1 Shinkawa, Chuo-ku Tokyo, 104-0033, Japan
TEL. +81-3-3297-2575　FAX. +81-3-3297-2612
URL：http://www.nikkakyo.org/
URL for LRI : http://www.j-lri.org/