ヒトiPSレポーター細胞を用いたシグナルかく乱を指標とする発生毒性試験法

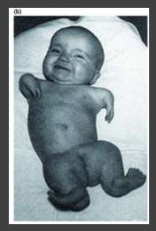
研究代表者　福田　淳二　　横浜国立大学
共同研究者　大久保　佑亮　国立医薬品食品衛生研究所
共同研究者　中島　芳浩　　産業技術総合研究所

Abstract

We have reported an approach for evaluating developmental toxicity through an FGF-SRF signal reporter assay utilizing human iPS cells. Signal interactions are vital for the regulation of fetal development. we hypothesized that developmental toxicity eventually relates to signal disruption, and thus established a signal reporter assay (DynaLux/c).

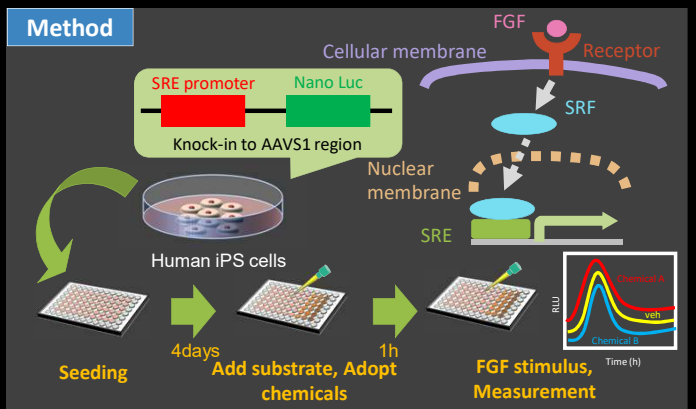
However, chemiluminescence was measured manually in this method. Thereby, it was difficult to capture detailed temporal changes and to measure during the night. Therefore, we automated luminescence measurements, establishing a method enabling detailed and prolonged luminescence assessments.

Malformations induced by Thalidomide



W.Rehman,.et al.,*Ther. Adv. Hematol.* 2, 291 (2011)

Method



Accuracy of this method

Tested Chemicals — False-negative

Developmental toxicity +		Developmental toxicity -	
5,5-Dimethyl-2,4-oxazolidinedione	Methotrexate hydrate*	Acetaminophen	Progesterone
5-Bromo-2'-deoxyuridine	Methoxyacetic acid	Acrylamide	Sodium saccharin
5-Fluorouracil*	Methylmercury chloride	Amoxicillin Trihydrate	Sulfasalazine
6-Aminonicotinamide	Misoprostol	Cefotaxime	
all-*trans*-Retinoic acid*	Phenytoin*	Cimetidine	
Boric acid	Pomalidomide*	D-Camphor	
Cyclophosphamide Monohydrate*	Sodium salicylate	Dimethyl phthalate	
Hydroxyurea*	(+/-)-Thalidomide*	Erythromycin	
Imatinib (mesylate)*	5-OH Thalidomide	Hydrochlorothiazide	
Lemalidomide	Valproic acid*	L-Ascorbic Acid	
Lithium chloride		Penicillin G sodium salt	

* ICH S5 (R3) Reference Compound Positive Control Examples for Qualifying Alternative Assays.

Accuracy：89%

Alternative Models in Developmental Toxicity Testing

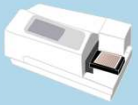
Model	Accuracy	References
Mouse embryonic stem cell test	78%	Genschow et al. (2002)
Rat MM test	70%	Genschow et al. (2002)
Rat WEC assay	80%	Genschow et al. (2002)
Zebrafish embryotoxicity test	72%	Chapin et al. (2008)
Frog embryo teratogenesis assay	NA	Bantle et al. (1989)

A. Luz,. et. al. *Toxicol. Sci.*. 165, 1 (2018)

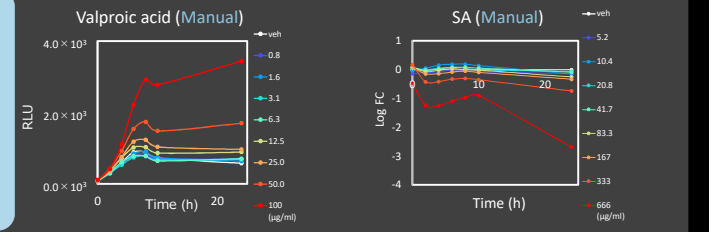
More accurate than existing methods

Automation of measurements

Previous method



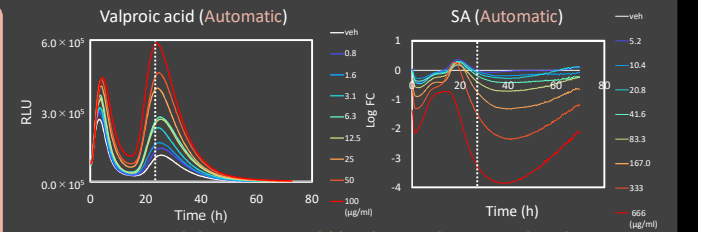
- ▲Manual
- ▲Fixed time points
  0, 2, 4, 6, 8, 10, 24h
- ▲Measuring time：24h



Current method

KronosHT

- Automatic
- Continuous monitor
- Measuring time：70h



Signal disruption could be detected in more detail

Reference

1. S. Kanno, Y. Okubo, T. Kageyama, L. Yan, S. Kitajima, and J. Fukuda, Establishment of a developmental toxicity assay based on human iPSC reporter to detect fibroblast growth factor signal disruption, ***iScience***, 25, 2, 103770 (2022)
2. S. Kanno, Y. Okubo, T. Kageyama, L. Yan, J. Fukuda, Integrated FGF signal disruptions in human iPS cells for prediction of teratogenic toxicity of chemicals, ***Journal of Bioscience and Bioengineering***, 133, 3, 291-299 (2022)
3. S. Kanno, K. Mizota, Y. Okubo, T. Kageyama, L. Yan, J. Fukuda, Luciferase assay system to monitor fibroblast growth factor (FGF) signal disruption in human iPSCs, ***Star Protocols***, 3, 2, 101439 (2022)
4. Y. Okubo, Y. Hirabayashi, J. Fukuda, Advances in Genomic Toxicology: In vitro Developmental Toxicity Test based on Signal Network Disruption Dynamics, ***Current Opinion in Toxicology***, in press