Title of Research:

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Development of an alternative method for teratogenicity using zebrafish

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Summary of Research:

Various attempts have been made to use zebrafish as a novel approach to current developmental toxicity testing. However, a standardized test protocol has not yet been established, and concerns remain regarding reproducibility and reliability, such as trial variability, strain differences, and inter-laboratory inconsistencies. This study aims to develop an alternative method for teratogenicity assessment using zebrafish and to utilize it for identifying adverse outcome pathways (AOPs). Initially, the principal investigator established a developmental toxicity testing protocol using zebrafish and then addressed the issue of strain differences. By decoding the whole genome sequences of zebrafish strains, their genetic relationships were clarified, and the strain-specific differences in toxic responses were investigated. The results revealed no significant strain differences in teratogenicity based on malformation observations following exposure to six compounds or in gene expression responses analyzed through RNA-Seq across low to high concentrations of three compounds, even among genetically distinct strains. Based on these findings, it was concluded that teratogenicity assessmentusing zebrafish provides reproducible and reliable test results, irrespective of the strain utilized. One of the key advantages of zebrafish-based developmental toxicity testing is the simplicity of conducting AOP analysis. By leveraging the whole-organism RNA-Seq technique developed during the strain difference analysis, AOP analyses focusing on gene expression were conducted. Multiple universal toxicity response genes, whose expression consistently decreased across exposures to several compounds, were identified. Moreover, a detailed time-course analysis following valproic acid exposure revealed early gene expression changes, including the downregulation of genes related to erythrocyte function and reactive oxygen species (ROS) elimination. These findings led to the hypothesis that key events in valproic acid-induced developmental teratogenicity include early-stage anemia and oxidative stress, ultimately resulting in energy deficits and oxidative damage. These mechanisms affect specific tissue development, culminating in teratogenicity.

Timeline:

March 1, 2023 – February 28, 2025

Topics:

1. "Development of an alternative method for teratogenicity using zebrafish" at the 2023 LRI Research report meeting of the Japan Chemical Industry Association. August 25, 2023.
2. "Development of an alternative method for teratogenicity using zebrafish" at the 2024 LRI Research report meeting of the Japan Chemical Industry Association. August 23, 2024.

Publications:

1. Kenichiro Sadamitsu, Fabien Velilla, Minori Shinya, Makoto Kashima, Yukiko Imai, Toshihiro Kawasaki, Kenta Watai, Miho Hosaka, **Hiromi Hirata*** and Noriyoshi Sakai*. (2024)

Establishment of a zebrafish inbred strain, M-AB, capable of regular breeding and genetic manipulation. Sci. Rep. 14(1): 7455. (*Corresponding authors)
2. Kanako Mori, Yoshinobu Aoki, Fumito Mikashima, Kazushige Maki, Toshio Tanaka, Mai Hayashi, Wataru Sugimoto, Mizuho Ono, Saaya Umekita, Tatsuhiro Niino, Michio Fujiwara, Tomonori Ebata, **Hiromi Hirata** and Hajime Kojima. (2024) Validation of a new protocol for a zebrafish MEFL (malformation or embryo-fetal lethality) test method that conforms to the ICH S5 (R3) guideline. J. Tox. Sci. 49(8): 337-348.
3. Kenichiro Sadamitsu, Kumiko Yanagi, Yuiko Hasegawa, Yoshiko Murakami, Sean E. Low, Daikun Ooshima, Yoichi Matsubara, Nobuhiko Okamoto, Tadashi Kaname* and **Hiromi Hirata***. (2024) A novel homozygous variant of the PIGK gene caused by paternal disomy in a patient with neurodevelopmental disorder, cerebellar atrophy, and seizures. J. Hum. Genet. 69: 553-563. (*Corresponding authors)
4. **Hiromi Hirata***, Tsuyoshi Tezuka and Kota Ujibe. (2024) Aging and Senescence Studies in Human and Zebrafish. Gerontology as an Interdisciplinary Science (Current Topics in Environmental Health and Preventive Medicine). Springer. Edited by Shiozawa, T., Hirata, H., Inoue, T., Kanikowska, D. and Takada, H. p3-22. (*Corresponding author)
5. Kenta Watai, Kenichiro Sadamitsu, Seiji Wada, Makoto Kashima and **Hiromi Hirata***. (2024) Zebrafish trpm7 mutants show reduced motility in free movement. Dev. Growth Differ. 66 (6): 349-356. (*Corresponding author)
6. Kenichiro Sadamitsu, Makoto Kashima, Seiji Wada, Akiko Ishioka, Satomi Nakayama, Ryoko Nakayama, Hitoshi Okamoto* and **Hiromi Hirata***. Establishment and genetic characterization of zebrafish RW line. Sci. Rep. Under Revision. (*Corresponding authors)