

Title of Research:23-1-03

Development of an alternative method for teratogenicity using zebrafish

Principal Investigator:

Hiromi Hirata, PhD (Professor, Department of Chemistry and Biological Science, College of Science and Engineering, Aoyama Gakuin University)
5-10-1 Fuchinobe, Sagamihara 252-5258, Japan
(tel) 042-759-6235 (email) hihirata@chem.aoyama.ac.jp

Collaborators:

Chitose Taya (Research Associate, Aoyama Gakuin University)
Kota Ujibe (Graduate Student, Aoyama Gakuin University)
Kenichiro Sadamitsu (Graduate Student, Aoyama Gakuin University)
Makoto Kashima (Lecturer, Toho University)
Junichi Tasaki (Kao Corporation)
Shujie Liu (Kao Corporation)

Summary of Research:

The development of alternatives to animal testing based on animal welfare principles is an urgent global priority. Zebrafish embryos before the onset of feeding are promising models because they fall outside many regulatory restrictions while retaining developmental mechanisms conserved with humans. This study established a reproducible teratogenicity testing platform using zebrafish embryos. A simplified exposure protocol, including embryo selection at 5 hours post-fertilization and a 24-well plate assay without medium replacement, reduced natural mortality and enabled stable results. Comparative genomic and transcriptomic analyses across multiple strains showed that strain-dependent differences in teratogenic sensitivity were minimal. Single-embryo RNA sequencing revealed early transcriptomic responses before visible malformations, identifying hypoxia and oxidative stress as key events in developmental toxicity.

Timeline:

March 2025 – February 2026

Topics:

Presented at the LRI annual research meeting of JCIA. August 29, 2025.

Publications:

1. Sadamitsu, K., Velilla, F., Shinya, M., Kashima, M., Imai, Y., Kawasaki, T., Watai, K., Hosaka, M., Hirata, H. and Sakai, N. (2024) Establishment of a zebrafish inbred strain, M-AB, capable of regular breeding and genetic manipulation. *Sci. Rep.* 14(1): 7455.
2. Mori, K., Aoki, Y., Mikashima, F., Maki, K., Tanaka, T., Hayashi, M., Sugimoto, W., Ono, M., Umekita, S., Niino, T., Fujiwara, M., Ebata, T., Hirata, H. and Kojima, H. (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. Toxicol. Sci.* 49(8): 337-348.
3. Sadamitsu, K., Kashima, M., Wada, S., Ishioka, A., Nakayama, S., Nakayama, R., Okamoto, H. and Hirata, H. (2025) Establishment and genetic characterization of zebrafish RW line. *Sci. Rep.* 15: 14512.
4. Mori, K., Aoki, Y., Hayashi, M., Sugimoto, W., Ono, M., Umekita, S., Niino, T., Ebata, T., Mikashima, F., Maki, K., Tanaka, T., Hirata, H. and Kojima, H. (2025) Variation and classification of chemically-induced zebrafish malformations for the ICH S5 (R3) guideline: an atlas for zebrafish teratogenesis. *J. Toxicol. Sci.* 50(8): 431-444.
5. Taya, C., Ujibe, K., Shimodaira, S., Sakamoto, A., Wada, S., Kashima, H. and Hirata, H. (2025) Comparative analysis of teratogen-induced malformations and gene expression across zebrafish strains in early development. *Toxicol. Rep.* 15: 102117.