Title of Research:

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

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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.