SciMedCentral

Annals of Marine Biology and Research

Perspective

Transgenic ZebraFish can Serve as Bioindicators of Environmental Toxicants at the Cellular Level

Huai-Jen Tsai*

Institute of Biomedical Sciences, Mackay Medical College, Taiwan

***Corresponding author**

Huai-JenTsai, Institute of Biomedical Sciences, Mackay Medical College, Taiwan, Email: hjtsai@ntu.edu.tw

Submitted: 26 April 2017

Accepted: 07 June 2017

Published: 11 June 2017

ISSN: 2573-105X

OPEN ACCESS

Nowadays, aromatic hydrocarbons, heavy metals, pesticides and endocrine disruptors are the most common environmental pollutants. Typically, chemical analysis is used to detect these toxicants. However, chemical analysis can only be applied to known toxicants using standard procedures and expensive equipment. Additionally, data obtained from chemical analysis does not faithfully reflect the physiological conditions of living organisms, such as stress at the cellular level. This calls for the development of an animal model with bio-sensitivity to a variety of subtle cellular and physiological changes upon exposure to environmental pollutants in the ecosystem.

Fish have long been used in toxicity assays [1]. Growth rate, survival rate, egg hatchability, mortality rate, abnormality rate, delayed or retarded embryonic development and enzymatic defenses of fish samples are frequent parameters used to evaluate toxicity. Nevertheless, subtle cellular and physiological damage cannot be assessed by such assays, and experimental fish must be sacrificed in order to collect samples for analysis. Therefore, the use of transgenic fish could be an alternative for monitoring environmental toxicity.

Owing to such merits as fecundity, transparency, light-controllable ovulation, easy gene manipulation, short maturation time, genetic tractability and compatibility with high-throughput screens, zebrafish (Danio rerio) and medaka (Oryziaslatipes) have become powerful model fish for use in academia and industry. More specifically, transgenic model fish are promising organisms for detecting environmental toxicants and mutagens [2,3].

Inducible promoters are key elements for the generation of transgenic fish with the bio-sensitivity required for a broad range of toxic substances. For example, cyp1a1 (encodes a member of the cytochrome P450 super family of enzymes) promoter, which is chemically induced [4], and heat-shock promoter, which is induced by heat [5], have been applied to generate transgenic fish [6], reported that environmental xenobiotics could be detected by transgenic medakahar boring GFP reporter driven by cytochrome P4501a promoter (CYP1A-GFP). Furthermore, xenoestrogenic compounds, such as PCBs, BPA and phthalates, can be specifically detected by a hybrid derived from crossing transgenic lines CYP1A-GFP and VG-Lux whose Lux reporter is driven by a fish vitellogenin promoter [7,8], reported the transgenic zebrafish Tg (cyp1a:gfp). Cyp1a is involved in the aryl hydrocarbon receptor pathway, and it can be induced by dioxins/dioxin-like compounds and polycyclic aromatic hydrocarbons, suggesting this transgenic fish could serve as a bio indicator for screening xenobiotic compounds. Estrogen response elements (EREs) have been also applied for detection of environmental estrogen [9,10]. However, in order for fish to serve as bio indicators of environmental pollutants, such promoters are either leaky expression, too sensitive or too specific. To explain, fish may encounter a stress condition that does notrise to the level of harming embryo development, but does induce reporter gene expression controlled by heat-shock promoter. Fish may also encounter stresses unrelated to aquatic pollutants, but such encounter still induces the expression of reporter gene driven by cyp1a1 promoter. In these cases, reporter expression would, in effect, sound an alarm that could be entirely unrelated to actual physiological disturbances, if any, caused by the respective stresses. Moreover, transgenic fish reported in these studies cannot indicate the level of toxicity which is actually attributed cellular stresses. In contrast, some promoters are too inert and will, therefore, not reflect any physiological response to surrounding environmental pollutants. Finally, in transgenic fish, selective promoters are not activated in cases of weak stress, which are precisely those stresses at the cellular level that result in chronic disease. More importantly, the inducible responsiveness of a promoter served for environmental toxicant's Bioindicators should not be specific for a particular type of compounds since environmental pollutants include aromatic hydrocarbons, heavy metals, pesticides and endocrine disruptors. Therefore, selection of a promoter with proper sensitivity against wide-spectrum pollutants is a key determinant for generating transgenic model fish used as Bioindicators to detect environmental toxicants causing cellular stress.

To address this point more directly, during stress, the endoplasmic reticulum (ER) triggers the unfolded protein response to regulate downstream gene expression and overcome ER stress before affected cells are induced to apoptosis [11]. Protein kinase RNA-like endoplasmic reticulum kinase (PERK) activation causes the phosphorylation of eIF2αand promotes the production of activating transcription factor 4 (ATF4),

leading, in turn, to increased expression of its downstream target CCAAT-enhancer-binding protein (C/EBP) homologous protein (CHOP) [12]. Thus, although PERK blocks global translation, the proapoptotic protein CHOP can be translated through by passing an inhibitory upper open reading frame (uORF) under stress. During lond-term ER stress, the transcription of chopis up regulated by ATF4, resulting in the decrease of anti-apoptotic Bcl2 family [13]. On the other hand, during a short-term ER stress, the up regulated chop can promote cell survival [14], a fact supported by [16], who demonstrated that the increase of CHOP enhances cultured neuronal cells against hypoxia-induced death. Therefore, CHOP is a marker protein which is able to monitor the absence or presence of cellular ER stresses. Interestingly [16], reported a zebrafish transgenic line, termed huORFZ, which harbors an uORF fragment of the human chop gene (huORFchop) fused with GFP reporter (huORFchop-gfp), but driven by cytomegalovirus promoter. In the absence of stress, the translation of downstream coding sequence (DCS) such as gfpmRNA in huORFZ embryos is completely blocked by huORFchop motif, resulting in no leaked GFP signal could be observed. Furthermore [17], demonstrated when the huORFZ embryos are under ER stresses, such as they are exposure to heat shock, cold shock, hypoxia, alcohol, heavy metals and hazardous contaminants near their sublethal concentrations (LC50), the translation of gfp mRNA located at DCS is initiated by the abolition of huORFchop-mediated translation inhibition, resulting in a different tissue-specific GFP expression patterns in embryos. Regarding sensitivity, huORFZ can easily detect pollutants with near LC10 concentration. The LOD of huORFZ can even be pushed to reach WHO guideline values for various heavy metals and EDCs. Regarding specificity, the mechanism of GFP signaling in huORFZ is not a specific response to target toxicants. Rather, it most likely reflects the level of physiological stress in the embryo's cells or tissues. Regarding reproducibility, individual variation does exist among huORFZ embryos. However, in most the cases, more than 70% of the embryos were responsive and exhibited similar GFP patterns. Collectively, these results demonstrate that zebrafish transgenic line huORFZ generated by Tsai's Lab at National Taiwan University can be an excellent Bioindicators candidate for environmental toxicants. More importantly, transgenic zebra fish huORFZ can be served as a first-line alarm system to detect the presence of stress-inducing chemicals, even when the chemical is unknown and not included in the standard water quality guidelines. Also, huORFZ embryos can be used to assess the effects of pollutants on living organisms exposed to chronic stress significantly below lethal dosages. Therefore, transgenic zebra fish huORFZ provides a suitable and handy animal model served as bioindicator for any stress occurring at cellular level induced by environmental toxicants because huORFZ meets the sensitivity requires, as described above, with its ability to respond to a broad range of pollutants of traceable cellular ER stresses. In summary, we demonstrated that the huORFchop-based system can be integrated into a first-line water security system monitoring water bodies contaminating environmental toxicants.

REFERENCES

1. Cardwell RD, Foreman DG, Payne TR, Wilbur DJ. Acute toxicity of selenium dioxide to freshwater fishes. Arch Environ Contam Toxicol. 1976; 4: 129-144.

2. Amanuma K, Takeda H, Amanuma H, Aoki Y. Transgenic zebrafish for detecting mutations caused by compounds in aquatic environments. Nat Biotechnol. 2000; 18: 62-65.

3. Amanuma K, Tone S, Saito H, Shigeoka T, Aoki Y. Mutational spectra of benzo[a] pyrene and MeIQx in rpsL transgenic zebrafish embryos. Mutat Res. 2002; 513: 83-92.

4. Wu YL, Pan X, Mudumana SP, Wang H, Kee PW, Gong Z. Development of a heat shock inducible gfp transgenic zebrafish line by using the zebrafish hsp27 promoter. Gene. 2008; 408: 85-94.

5. Halloran MC, Sato-Maeda M, Warren JT, Su F, Lele Z, Krone PH, et al. Laser-induced gene expression in specific cells of transgenic zebrafish. Development. 2000; 127: 1953-1960.

6. Chen T, Lu JK, De la Fuente J, Castro FO. Transgenic fish technology: basic principles and their application in basic and applied research. Gene Transfer in Aquatic Organism. Berlin: Springer; 1998; 45-73.

7. Ng GH, Gong Z. GFP transgenic medaka (Oryziaslatipes) under the inducible cyp1a promoter provide a sensitive and convenient biological indicator for the presence of TCDD and other persistent organic chemicals. PLoS One. 2013; 8: 64334

8. Xu H, Li C, Li Y, Ng GH, Liu C, Zhang X, et al. Generation of Tg (cyp1a:gfp) transgenic zebrafish for development of a convenient and sensitive in vivo assay for aryl hydrocarbon receptor activity. Mar Biotechnol (NY). 2015; 17: 831-840.

9. Chen H, Hu J, Yang J, Wang Y, Xu H, Jiang Q, et al. Generation of a fluorescent transgenic zebrafish for detection of environmental estrogens. Aquat Toxicol. 2010; 96: 53-61.

10. Lee O, Takesono A, Tada M, Tyler CR, Kudoh T. Biosensor zebrafish provide new insights into potential health effects of environmental estrogens. Environ Health Persp. 2012; 120: 990-996.

11. Winnay JN, Kahn CR. PI 3-kinase regulatory subunits as regulators of the unfolded protein response. Methods Enzymol. 2011; 490: 147-158.

12. Ron D, Habener JF. CHOP, a novel developmentally regulated nuclear protein that dimerizes with transcription factors C/EBP and LAP and functions as a dominant-negative inhibitor of gene transcription. Genes Dev. 1992; 6: 439-453.

13. Harding HP, Zhang Y, Bertolotti A, Zeng H, Ron D. Perk is essential for translational regulation and cell survival during the unfolded protein response. Mol Cell. 2000; 5: 897-904.

14. Skalet AH, Isler JA, King LB, Harding HP, Ron D, Monroe JG. Rapid B cell receptor-induced unfolded protein response in nonsecretory B cells correlates with pro- versus antiapoptotic cell fate. J BiolChem. 2005; 280: 39762-39771.

15. Halterman MW, Gill M, DeJesus C, Ogihara M, Schor NF, Federoff HJ. The endoplasmic reticulum stress response factor CHOP-10 protects against hypoxia-induced neuronal death. J BiolChem. 2010; 285: 21329-21340.

16. Lee HC, Chen YJ, Liu YW, Lin KY, Chen SW, Lin CY, et al. Transgenic zebrafish model to study translational control mediated by upstream open reading frame of human chop gene. Nucleic Acids Res. 2011; 39: 139.

17. Lee HC, Lu PN, Huang HL, Chu C, Li HP, Tsai HJ. Zebrafish transgenic line huORFZ is an effective living bioindicator for detecting environmental toxicants. PLoS One. 2014; 9: 90160.

Cite this article

Tsai HJ (2017) Transgenic ZebraFish can Serve as Bioindicators of Environmental Toxicants at the Cellular Level. Ann Mar Biol Res 4(2): 1022.