Editorial Commentary

BIO Integration

Utilization of Zebrafish as a Model System in Medical Research

Sanxiong Liu[1,*]

Introduction

Basic medical research relies on animal models to advance understanding of the pathogenesis of human diseases and enable the discovery of innovative treatments [1]. Although rodents are the most widely used model organisms, in recent decades, zebrafish have become an important model in biomedical research [2, 3]. The first genetic studies in zebrafish combined chemical mutagenesis through N-ethyl-N-nitrosourea [4] with phenotypic screening [5, 6].

As a lower-vertebrate model, zebrafish offer many advantages over other higher vertebrates in modeling vertebrate development and disease [7, 8] (**Figure 1**), including their easy maintenance, high reproductive rate, transparent embryos that develop rapidly and externally, and fully sequenced genome [9, 10]. In addition, advanced gene targeting technologies, including CRISPR/Cas9, have greatly facilitated the generation of specific gene knockout or knock-in mutations in zebrafish [11–13]. The availability of more than 10,000 mutant strains in zebrafish [14] is another benefit of this model species. Here, I discuss the recent progress in, and potential of, using zebrafish for modeling human diseases—particularly cancers, neurological and metabolic diseases, and several rare diseases [15]—as well as discovering new drugs (**Table 1**).

Zebrafish in modeling human diseases

Developmental disorders

The advantage of externally developing, transparent embryos makes zebrafish an attractive model for investigating mammalian development. The accessibility of zebrafish genetic manipulation in various experimental paradigms is exemplified in the modeling of neurodevelopmental disorders (NDDs). For an increased number of zebrafish genes, their orthologs in human have been associated with NDDs further supporting zebrafish for modeling NDDs [16–18]; however, the whole-genome duplication in teleosts adds complexity to this process [14]. Zebrafish also present conserved and complexed neuronal subtypes, including GABAergic, monoaminergic, purinergic, glutamatergic and melatonergic systems [19–21]. In addition, the high neuroplasticity in the zebrafish central nervous system enables the analysis of neuronal adaptations and their behavioral output, as controlled by relatively well-defined circuits [22, 23]. The abilities to perform microscopy [24], and to engineer the expression of voltage or calcium sensors [25] and multiple fluorescent proteins [26] in distinct neurons greatly facilitate visualizing, recording and manipulating neuronal activity in defined circuits in control and disease states. Collectively, these advantages have enabled investigations of underlying mechanisms and potential therapies for neurological disorders. For example, zebrafish have been used to model multiple neurodevelopmental disorders, including autism spectrum disorder [17], developmental delay, attention deficit/hyperactivity disorder [16] and intellectual disability [27].

Cancer

Zebrafish have several attributes that make them a widely used model for investigating cancer biology and metastasis. Zebrafish and mammals share conserved molecular pathways involved in tumor progression [48]. Likewise, many cell-cycle genes, oncogenes and tumor suppressors are conserved [49] between zebrafish and mammals, thus allowing tumorigenic signatures to be studied in zebrafish. Although zebrafish have a very low rate of spontaneous tumorigenesis, they respond to exposure to carcinogenic

[1]Department of Biochemistry and Molecular Pharmacology, New York University Grossman School of Medicine, New York, NY 10016, USA

*Correspondence to:
Sanxiong Liu,
E-mail: sanxiong.liu@nyumc.org

Received: June 3 2022
Revised: July 11 2022
Accepted: July 19 2022
Published Online: August 1 2022

Available at:
https://bio-integration.org/

BIOI 2022, Vol 3, No. 4, 188–192
https://bio-integration.org doi: 10.15212/bioi-2022-0019

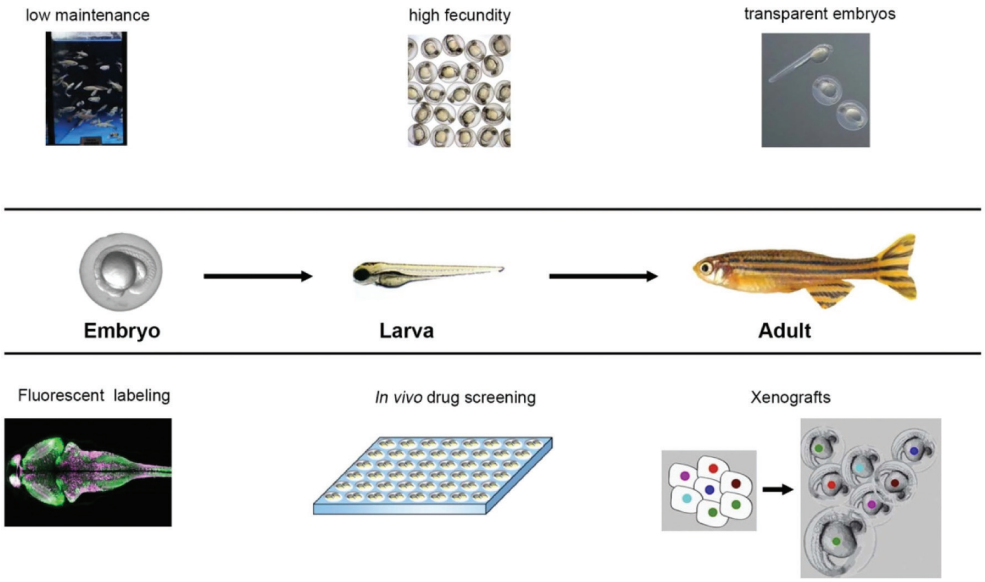

Figure 1 The advantages of zebrafish as a system for modeling human diseases and discovering innovative therapies.

Table 1 List of Current Medical Applications Using Zebrafish as the Model System

Application	Disease	
Disease modeling	Neurological	Autism spectrum disorder [17]
		Intellectual disability [27]
		Attention deficit/hyperactivity disorder [16]
		Alzheimer's disease [28]
		Parkinson's disease [29]
		Amyotrophic lateral sclerosis [30]
	Cancer	Leukemia [31]
		Melanoma [32]
		Pancreatic cancer [33]
		Liver cancer [34]
	Metabolic	Obesity [35]
		Diabetes [36]
		Atherosclerosis [37]
	Rare diseases	Diamond–Blackfan anemia [38]
		DiGeorge syndrome [39]
		COACH syndrome [40]
	Disease and identified drug	
Drug screening	Amyotrophic lateral sclerosis	TRVA242 [41]
	Dravet syndrome	Fenfluramine [42–44]
	Melanoma	Leflunomide [45]
	Adenoid cystic carcinoma	All-trans retinoic acid [46]
	Diamond–Blackfan anemia	Trifluoperazine [47]

agents, such as DENA, MNNG and DMBA, thus resulting in efficient cancer model development [50]. Zebrafish models of many cancers, such as leukemia [31], melanoma [32], pancreatic cancer [51] and liver cancer, have been generated and studied. More importantly, zebrafish embryos can be transplanted with human tumor cells to facilitate the investigation of tumor cell migration, metastasis and angiogenesis [52].

Metabolic diseases

Defects in converting food to energy in the body result in various metabolic disorders. The balance between energy production and expenditure is achieved through a cooperation among several organs, including the brain, heart, intestines, liver, skeletal muscle, kidneys and adipose tissue. Thus, animal models are preferred over cell-culture systems to study the entire process of metabolism. Although zebrafish and humans differ in their food and nutrient requirements, the similarity in adipocyte anatomy between zebrafish and mammals, and the presence of all essential organs required for metabolism make zebrafish a suitable system for studying diabetes, obesity, adipogenesis and other metabolic diseases [53, 54]. Furthermore, the microbiome—which has been shown to be involved in obesity and obesity-associated diseases in mammals [55]—can be easily modulated in zebrafish [56, 57]. For all these reasons, various approaches,

such as diet-induced, transgenic and/or mutant models, are being generated in zebrafish to study the molecular pathways involved in the development and progression of obesity [35], diabetes [36] and lipid-associated diseases [58].

Zebrafish in drug discovery and toxicology

The permeability of zebrafish embryos to many small molecules is critical for chemical screening [59], although other properties of small-molecule drugs must be considered, including Lipinski's rule of five, solubility, logP and pH. Small molecules can be added to the plates used to culture zebrafish embryos, thus enabling high-throughput chemical screening of tens of thousands of compounds in living embryos [60]. The ability to perform drug screening in zebrafish as whole animals overcomes several hurdles in current drug discovery which relies on in vitro cell lines. Moreover, it offers a valuable toxicity testing platform simultaneously. By performing drug screening in transgenic or mutant zebrafish with particular disease phenotypes, compounds that suppress specific diseases can be identified [61]. Moreover, zebrafish provide 3Rs (replacement, refinement and reduction) value in drug-discovery and toxicology studies [61]. In recent years, zebrafish have become the preferred animal model for high-throughput screening of chemical drugs targeting various human diseases [62]. Numerous compounds identified by drug screening in zebrafish have recently entered clinical trials, for example, leflunomide for melanoma, all-trans retinoic acid for adenoid cystic carcinoma, fenfluramine for Dravet syndrome and trifluoperazine for Diamond–Blackfan anemia [63]. However, drugs identified through screening in zebrafish should be retested in multiple laboratories worldwide to confirm the validity of the findings [15].

Summary

In summary, zebrafish are a very powerful vertebrate animal model in biomedical research in human disease modeling and drug discovery. With advances in CRISPR/Cas9-mediated genome editing and informative data from next-generation sequencing, disease models in zebrafish offer unique opportunities to enhance accurate understanding of the pathogenesis of human diseases, and develop innovative and effective treatments against a wide range of human diseases modeled in zebrafish. Because differences exist between zebrafish and humans in terms of disease pathogenesis and drug treatment, translating drugs identified in zebrafish models into clinical use remains challenging. This limitation may be addressed through use of a combination of zebrafish and rodent models and/or other research methods [64]. Zebrafish will never replace rodents in later phases of drug discovery, but can serve as a highly useful complementary model system in earlier phases.

References

[1] Maurer KJ, Quimby FW. Chapter 34 - animal models in biomedical research. In: Fox JG, Anderson LC, Otto GM, Pritchett-Corning KR, Whary MT, editors. Laboratory animal medicine. 3rd ed. Academic Press; 2015. pp. 1497-534. [DOI: 10.1016/B978-0-12-409527-4.00034-1].

[2] Choi TY, Choi TI, Lee YR, Choe SK, Kim CH. Zebrafish as an animal model for biomedical research. Exp Mol Med 2021;53:310-7. [PMID: 33649498 DOI: 10.1038/s12276-021-00571-5].

[3] Teame T, Zhang Z, Ran C, Zhang H, Yang Y, et al. The use of zebrafish (Danio rerio) as biomedical models. Anim Front 2019;9:68-77. [PMID: 32002264 DOI: 10.1093/af/vfz020]

[4] Streisinger G, Walker C, Dower N, Knauber D, Singer F. Production of clones of homozygous diploid zebra fish (Brachydanio rerio). Nature 1981;291:293-6. [PMID: 7248006 DOI: 10.1038/291293a0]

[5] Kimmel CB. Genetics and early development of zebrafish. Trends Genet 1989;5:283-8. [PMID: 2686119 DOI: 10.1016/0168-9525(89)90103-0]

[6] Grunwald DJ, Streisinger G. Induction of recessive lethal and specific locus mutations in the zebrafish with ethyl nitrosourea. Genet Res 1992;59:103-16. [PMID: 1628817 DOI: 10.1017/s0016672300030317]

[7] Dahm R, Geisler R. Learning from small fry: the Zebrafish as a genetic model organism for aquaculture fish species. Mar Biotechnol 2006;8:329-45. [PMID: 16670967 DOI: 10.1007/s10126-006-5139-0]

[8] Kari G, Rodeck U, Dicker AP. Zebrafish: an emerging model system for human disease and drug discovery. Clin Pharmacol Ther 2007;82:70-80. [PMID: 17495877 DOI: 10.1038/sj.clpt.6100223]

[9] Lieschke GJ, Currie PD. Animal models of human disease: zebrafish swim into view. Nat Rev Genet 2007;8:353-67. [DOI: 10.1038/nrg2091]

[10] Shin JT, Fishman MC. From Zebrafish to Human: modular medical models. Annu Rev Genomics Hum Genet 2002;3:311-40. [PMID: 12142362 DOI: 10.1146/annurev.genom.3.031402.131506]

[11] Sung YH, Kim JM, Kim HT, Lee J, Jeon J, et al. Highly efficient gene knockout in mice and zebrafish with RNA-guided endonucleases. Genome Res 2014;24:125-31. [PMID: 24253447 DOI: 10.1101/gr.163394.113]

[12] Auer TO, Duroure K, Cian AD, Concordet J-P, Bene FD. Highly efficient CRISPR/Cas9-mediated knock-in in zebrafish by homology-independent DNA repair. Genome Res 2014;24:142-53. [PMID: 24253447 DOI: 10.1101/gr.163394.113]

[13] Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, et al. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat Biotechnol 2013;31:227-9. [PMID: 23360964 DOI: 10.1038/nbt.2501]

[14] Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, et al. The zebrafish reference genome sequence and its relationship to the human genome. Nature 2013;496:498-503. [PMID: 23594743 DOI: 10.1038/nature12111]

[15] Crouzier L, Richard EM, Sourbron J, Lagae L, Maurice T, et al. Use of zebrafish models to boost research in rare genetic diseases. Int J Mol Sci 2021;22:13356. [PMID: 34948153 DOI: 10.3390/ijms222413356]

[16] Meshalkina DA, Kizlyk MN, Kysil EV, Collier AD, Echevarria DJ, et al. Zebrafish models of autism spectrum disorder. Exp Neurol 2018;299:207-16. [PMID: 28163161 DOI: 10.1016/j.expneurol.2017.02.004]

[17] Fernandes Y, Tran S, Abraham E, Gerlai R. Embryonic alcohol exposure impairs associative learning performance in adult zebrafish. Beh Brain Res 2014;265:181-7. [PMID: 24594368 DOI: 10.1016/j.bbr.2014.02.035]

[18] Fontana BD, Franscescon F, Rosemberg DB, Norton WHJ, Kalueff AV, et al. Zebrafish models for attention deficit hyperactivity disorder (ADHD). Neurosc Biobehav Rev 2019;100:9-18. [PMID: 30779935 DOI: 10.1016/j.neubiorev.2019.02.009]

[19] Bhattarai P, Thomas AK, Zhang Y, Kizil C. The effects of aging on Amyloid-β42-induced neurodegeneration and regeneration in adult zebrafish brain. Neurogenesis 2017;4:e1322666. [PMID: 28656156 DOI: 10.1080/23262133.2017.1322666]

[20] Brown SJ, Boussaad I, Jarazo J, Fitzgerald J, Antony P, et al. PINK1 deficiency impairs adult neurogenesis of dopaminergic neurons. Sci Rep 2021;11:6617. [PMID: 33758225 DOI: 10.1038/s41598-021-84278-7]

[21] Campanari ML, Marian A, Ciura S, Kabashi E. TDP-43 regulation of AChE expression can mediate ALS-like phenotype in zebrafish. Cells 2021;10:221. [PMID: 33499374 DOI: 10.3390/cells10020221]

[22] Teittinen KJ, Grönroos T, Parikka M, Rämet M, Lohi O. The zebrafish as a tool in leukemia research. Leuk Res 2012;36:1082-8. [PMID: 22749067 DOI: 10.1016/j.leukres.2012.06.001]

[23] Dovey M, White RM, Zon LI. Oncogenic NRAS cooperates with p53 Loss to generate melanoma in zebrafish. Zebrafish 2009;6:397-404. [PMID: 19954345 DOI: 10.1089/zeb.2009.0606]

[24] Park JT, Leach SD. Zebrafish model of KRAS-initiated pancreatic cancer. Anim Cells Syst (Seoul) 2018;22:353-9. [PMID: 30533257 DOI: 10.1080/19768354.2018.1530301]

[25] Lee AQ, Li Y, Gong Z. Inducible liver cancer models in transgenic zebrafish to investigate cancer biology. Cancers 2021;13:5148. [PMID: 34680297 DOI: 10.3390/cancers13205148]

[26] Khanal P, Patil BM, Unger BS. Zebrafish shares common metabolic pathways with mammalian olanzapine-induced obesity. Futur J Pharm Sci 2020;6:36. [DOI: 10.1186/s43094-020-00049-7]

[27] Salehpour A, Rezaei M, Khoradmehr A, Tahamtani Y, Tamadon A. Which hyperglycemic model of zebrafish (Danio rerio) suites my type 2 diabetes mellitus research? A scoring system for available methods. Front Cell Dev Biol 2021;9:652061. [PMID: 33791308 DOI: 10.3389/fcell.2021.652061]

[28] Tang D, Geng F, Yu C, Zhang R. Recent application of zebrafish models in atherosclerosis research. Front Cell Dev Biol 2021;9:643697. [PMID: 33718384 DOI: 10.3389/fcell.2021.643697]

[29] Danilova N, Wilkes M, Bibikova E, Youn MY, Sakamoto KM, Lin S, et al. Innate immune system activation in zebrafish and cellular models of Diamond Blackfan Anemia. Sci Rep 2018;8:5165. [DOI: 10.1038/s41598-018-23561-6]

[30] Lopez-Rivera E, Liu Y, Verbitsky M, Anderson B, Capone V, et al. Genetic drivers of kidney defects in the DiGeorge syndrome. N Engl J Med 2017;376:742-54. [DOI: 10.1056/NEJMoa1609009]

[31] Lee SH, Nam TS, Li W, Kim JH, Yoon W, et al. Functional validation of novel MKS3/TMEM67 mutations in COACH syndrome. Sci Rep 2017;7:10222. [DOI: 10.1038/s41598-017-10652-z]

[32] Bose P, Tremblay E, Maios C, Narasimhan V, Armstrong GAB, et al. The novel small molecule TRVA242 stabilizes neuromuscular junction defects in multiple animal models of amyotrophic lateral sclerosis. Neurotherapeutics 2019;16:1149-66. [PMID: 31342410 DOI: 10.1007/s13311-019-00765-w]

[33] Sourbron J, Schneider H, Kecskés A, Liu Y, Buening EM, et al. Serotonergic modulation as effective treatment for dravet syndrome in a zebrafish mutant model. ACS Chem Neurosci 2016;7:588-98. [PMID: 26822114 DOI: 10.1021/acschemneuro.5b00342]

[34] Sourbron J, Smolders I, de Witte P, Lagae L. Pharmacological analysis of the anti-epileptic mechanisms of fenfluramine in scn1a mutant zebrafish. Front Pharmacol 2017;8:191. [PMID: 28428755 DOI: 10.3389/fphar.2017.00191]

[35] Sullivan J, Simmons R. Fenfluramine for treatment-resistant epilepsy in Dravet syndrome and other genetically mediated epilepsies. Drugs Today (Barc) 2021;57:449-54. [PMID: 34268532 DOI: 10.1358/dot.2021.57.7.3284619]

[36] White RM, Cech J, Ratanasirintrawoot S, Lin CY, Rahl PB, et al. DHODH modulates transcriptional elongation in the neural crest and melanoma. Nature 2011;471:518-22. [PMID: 21430780 DOI: 10.1038/nature09882]

[37] Mandelbaum J, Shestopalov IA, Henderson RE, Chau NG, Knoechel B, et al. Zebrafish blastomere screen identifies retinoic acid suppression of MYB in adenoid cystic carcinoma. J Exp Med 2018;215:2673-85. [PMID: 30209067 DOI: 10.1084/jem.20180939]

[38] Uechi T, Kenmochi N. Zebrafish models of diamond-blackfan anemia: a tool for understanding the disease pathogenesis and drug discovery. Pharmaceuticals (Basel) 2019;12:151. [PMID: 31600948 DOI: 10.3390/ph12040151]

[39] Vaz R, Hofmeister W, Lindstrand A. Zebrafish models of neurodevelopmental disorders: limitations and benefits of current tools and techniques. Int J Mol Sci 2019;20:1296. [PMID: 30875831 DOI: 10.3390/ijms20061296]

[40] Panula P, Sallinen V, Sundvik M, Kolehmainen J, Torkko V, et al. Modulatory neurotransmitter systems and behavior: towards zebrafish models of neurodegenerative diseases. Zebrafish 2006;3:235-47. [PMID: 18248264 DOI: 10.1089/zeb.2006.3.235]

[41] Genario R, Giacomini ACVVV, Demin KA, Santos BED, Marchiori NI, et al. The evolutionarily conserved role of melatonin in CNS disorders and behavioral regulation: translational lessons from zebrafish. Neurosci Biobehav Rev 2019;99:117-27. [PMID: 30611799 DOI: 10.1016/j.neubiorev.2018.12.025]

[42] Roy B, Ali DW. Multiple types of GABAA responses identified from zebrafish Mauthner cells. NeuroReport 2014;25:1232-6. [PMID: 25162782 DOI: 10.1097/WNR.0000000000000258]

[43] Ghosh S, Hui SP. Regeneration of Zebrafish CNS: adult neurogenesis. Neural Plast 2016;2016:e5815439. [PMID: 27382491 DOI: 10.1155/2016/5815439]

[44] Perathoner S, Cordero-Maldonado ML, Crawford AD. Potential of zebrafish as a model for exploring the role of the amygdala in emotional memory and motivational behavior. J Neurosci Res 2016;94:445-62. [PMID: 26833658 DOI: 10.1002/jnr.23712]

[45] Hildebrand DGC, Cicconet M, Torres RM, Choi W, Quan TM, et al. Whole-brain serial-section electron microscopy in larval zebrafish. Nature 2017;545:345-9. [PMID: 28489821 DOI: 10.1038/nature22356]

[46] Kim DH, Kim J, Marques JC, Grama A, Hildebrand DGC, et al. Pan-neuronal calcium imaging with cellular resolution in freely swimming zebrafish. Nat Methods 2017;14:1107-14. [DOI: 10.1038/nmeth.4429]

[47] Pan YA, Freundlich T, Weissman TA, Schoppik D, Wang XC, et al. Zebrabow: multispectral cell labeling for cell tracing and lineage analysis in zebrafish. Development 2013;140:2835-46. [PMID: 23757414 DOI: 10.1242/dev.094631]

[48] Lam SH, Wu YL, Vega VB, Miller LD, Spitsbergen J, et al. Conservation of gene expression signatures between zebrafish and human liver tumors and tumor progression. Nat Biotechnol 2006;24:73-5. [PMID: 16327811 DOI: 10.1038/nbt1169]

[49] Zheng W, Li Z, Nguyen AT, Li C, Emelyanov A, et al. XMRK, KRAS and MYC transgenic zebrafish liver cancer models share molecular signatures with subsets of human hepatocellular carcinoma. PLoS One 2014;9:e91179. [PMID: 24633177 DOI: 10.1371/journal.pone.0091179]

[50] Spitsbergen JM, Tsai HW, Reddy A, Miller T, Arbogast D, et al. Neoplasia in zebrafish (Danio rerio) treated with N-methyl-N'-nitro-N-nitrosoguanidine by three exposure routes at different developmental stages. Toxicol Pathol 2000;28:716-25. [PMID: 11026608 DOI: 10.1177/019262330002800512]

[51] Liu S, Leach SD. Zebrafish models for cancer. Ann Rev Pathol: Mechanisms of Disease 2011;6:71-93. [PMID: 21261518 DOI: 10.1146/annurev-pathol-011110-130330]

[52] Tobia C, Sena GD, Presta M. Zebrafish embryo, a tool to study tumor angiogenesis. Int J Dev Biol. 2011;55:505-9. [PMID: 21858773 DOI: 10.1387/ijdb.103238ct]

[53] Seth A, Stemple DL, Barroso I. The emerging use of zebrafish to model metabolic disease. Dis Model Mech 2013;6:1080-8. [PMID: 24046387 DOI: 10.1242/dmm.011346]

[54] Asaoka Y, Terai S, Sakaida I, Nishina H. The expanding role of fish models in understanding non-alcoholic fatty liver disease. Dis Model Mech 2014;7:409. [PMID: 23720231 DOI: 10.1242/dmm.011981]

[55] Davis CD. The gut microbiome and its role in obesity. Nutr Today 2016;51:167-74. [PMID: 27795585 DOI: 10.1097/NT.0000000000000167]

[56] Cornuault JK, Byatt G, Paquet ME, De Koninck P, Moineau S. Zebrafish: a big fish in the study of the gut microbiota. Curr Opin Biotechnol 2022;73:308-13. [PMID: 34653834 DOI: 10.1016/j.copbio.2021.09.007]

[57] Lu H, Li P, Huang X, Wang CH, Li M, et al. Zebrafish model for human gut microbiome-related studies: advantages and limitations. Med Microecol 2021;8:100042. [DOI: 10.1016/j.medmic.2021.100042]

[58] Liang X, Cao S, Xie P, Hu X, Lin Y, et al. Three-dimensional imaging of whole-body zebrafish revealed lipid disorders associated with niemann-pick disease type C1. Anal Chem. 2021;93:8178-87. [PMID: 34061502 DOI: 10.1021/acs.analchem.1c00196]

[59] Peterson RT, Link BA, Dowling JE, Schreiber SL. Small molecule developmental screens reveal the logic and timing of vertebrate development. Proc Natl Acad Sci U S A 2000;97:12965-9. [PMID: 11087852 DOI: 10.1073/pnas.97.24.12965]

[60] Lubin A, Otterstrom J, Hoade Y, Bjedov I, Stead E, et al. A versatile, automated and high-throughput drug screening platform for zebrafish embryos. Biol Open 2021;10:bio058513. [PMID: 34472582 DOI: 10.1242/bio.058513]

[61] Cassar S. Adatto I, Freeman JL, Gamse JT, Iturria I, et al. Use of zebrafish in drug discovery toxicology. Chem Res Toxicol. 2020;33:95-118. [PMID: 31625720 DOI: 10.1021/acs.chemrestox.9b00335]

[62] Lee HC, Lin CY, Tsai HJ. Zebrafish, an in vivo platform to screen drugs and proteins for biomedical use. Pharmaceuticals 2021;14:500. [PMID: 34073947 DOI: 10.3390/ph14060500]

[63] Patton EE, Zon LI, Langenau DM. Zebrafish disease models in drug discovery: from preclinical modelling to clinical trials. Nat Rev Drug Discov 2021;20:611-28. [PMID: 34117457 DOI: 10.1038/s41573-021-00210-8]

[64] Langova V, Vales K, Horka P, Horacek J. The role of zebrafish and laboratory rodents in schizophrenia research. Front Psychiatry 2020;11:703. [PMID: 33101067 DOI: 10.3389/fpsyt.2020.00703]