COMMENTARY

Zebrafish embryos as *in vivo* test tubes to unravel cell-specific mechanisms of neurogenesis during neurodevelopment and in diseases

Éric Samarut

Research Center of the University of Montreal Hospital Center (CRCHUM), Department of Neurosciences, Université de Montréal, Montréal, QC, Canada

ABSTRACT

Zebrafish has become a model of choice for developmental studies in particular for studying neural development and related mechanisms involved in diseases. Indeed, zebrafish provides a fast, handy and accurate model to perform functional genomics on a gene or network of genes of interest. Recently, we successfully purified neural stem cells (NSCs) by fluorescence-activated cell sorting (FACS) from whole embryos in order to analyze cell-specific transcriptomic effects by RNA sequencing. As a result, our work sheds light on signaling pathways that are more likely to be involved in our morpholino-induced neurogenesis phenotype. This cell purification strategy brings zebrafish to a higher level since it now allows one to investigate cell-specific effects of a genetic condition of interest (knockout, knock-down, gain-of-function etc.) at the genomic, transcriptomic and proteomic levels in a genuine *in vivo* context. With this new potential, there is no doubt that zebrafish will be of a major model with which to unravel complex underlying molecular mechanisms of neurological disorders such as epilepsy, autism spectrum disorders and schizophrenia.

ARTICLE HISTORY

Received 25 May 2016
Revised 25 August 2016
Accepted 31 August 2016

KEYWORDS

CRISPR mutagenesis; FACS
cell sorting; proteomics;
transcriptomics; transgenesis;
zebrafish

Zebrafish ride the wave

In the last decade, zebrafish has emerged as a promising model to investigate biological issues such as developmental biology,^{1,2} physiology,^{3,4} evolutionary biology^{5,6} and toxicology³ and it is even used as a model to teach life sciences.⁷ Particularly, research investigations using zebrafish have been important to decipher basic developmental mechanisms of the central nervous system⁸⁻¹⁰ as well as to test for neurotoxic effects of chemicals.^{11,12}

The reasons for the popularity of zebrafish are numerous and external fertilization is one of the most important as it makes the embryo suitable for developmental observations from the earliest stages of development. Moreover, this is very convenient for genetic manipulation from the very first cell stage (Fig. 1). Indeed, by microinjecting molecular tools in the one-cell stage embryo, it is possible to either knockdown the expression of a gene of interest (using morpholino oligonucleotides) or to overexpress its expression (by injecting *in vitro* transcribed mRNAs). Although being subject to variability, this manual

micromanipulation of gene expression in the embryo can be considered as an advantage since it allows subtle dose-dependent injection shadings. Moreover, the transparency of the embryo allows one to follow *in vivo* organogenesis, in particular the observation of the central nervous system structures with a single-cell resolution. Indeed, single-cell behaviors can be monitored in time-lapse during neurodevelopment such as radial glial asymmetric division in the developing forebrain.¹³ Embryo development is very fast from a single-cell to larvae in one day and to an autonomous swimming larva in less than a week. Lastly, another main advantage of zebrafish is the large list of available transgenic lines expressing fluorescent reporters in different cell population (Fig. 1). In particular, numerous lines have been generated to specifically label neuronal cell population of interest such as post-mitotic neurons (*huc/elavl3+*),¹⁴ motoneurons (*hb9+*),¹⁵ interneurons (*evx+*),¹⁶ sensory neurons (*ngn1+*)¹⁷ or oligodendrocytes (*olig2+*).¹⁸ Moreover, the galactose-inducible-derived GAL4/UAS system is commonly used in zebrafish¹⁹ and its use expands

Figure 1. Zebrafish embryos as *in vivo* test tubes. Zebrafish provides many advantages such as genetic accessibility, fast development and transparency of the developing embryo. Using specific transgenic lines, we were able to dissociate our cell population of interest (e.g. neural stem cells) by FACS. A large panel of omics analysis (i.e. RNA sequencing) can subsequently be performed on *in vivo* purified cells.

endlessly the possibility to fluorescently identify specific cell population within the developing embryo. We will further highlight that this is of particular interest for using zebrafish for cell-specific genomic analysis. Altogether, this explains the top position at which zebrafish has been ranked by the scientific community, and in particular in the neuroscience field.

Fishing for deeper molecular mechanisms

Started in 2001, the zebrafish genome project continues to constantly improve genome annotation, the latest update being the GRCz10 version. Systematic functional genomic analysis has been initiated aiming at correlating disruption in gene expression with specific developmental phenotypes.²⁰ Moreover, a recent comparison with the human genome showed that 70% of human genes have at least one obvious zebrafish orthologous therefore validating the use of zebrafish to investigate human protein function and associated genetic diseases.^{21,22}

As a result, although being widely used as a model to investigate developmental issues, zebrafish also

emerged as a promising model to investigate deeper molecular mechanisms hidden behind the observed phenotype. Indeed, thanks to the quality and the accessibility of the zebrafish genome, transcriptomics and genomics analysis are now facilitated and affordable.²³ As a result, studies have taken advantage of these techniques in order to test the effect of compounds or of morpholino gene knockdown on the overall transcriptome of the early zebrafish embryos.^{24,25} In these studies, the fact that sequenced RNAs were extracted from whole embryos could be taken as a drawback when considering that some subtle changes in gene expression could only occur in discrete cell populations within the developing embryo. As a result, whole embryo extraction could dilute the specificity of the signal and prevent the identification of sensitive changes in gene expression. To circumvent this problem, the dissection of a specific organ such as the heart allows improving the specificity of the transcriptomic analysis by focusing specifically in the tissue of interest.²⁶ However, dissection of tiny structures can be tricky to achieve accurately but more importantly, it does not allow the isolation of specific

cell populations within a tissue like neurons. Other studies used cell-specific chemical ablation to selectively kill the cell population of interest (i.e. cranial sensory neurons).²⁷ Consequently, by comparing the transcriptome of cell-depleted versus wild-type embryos, this offered an indirect access to the differential gene expression that is assumed to be specific to the depleted cells.

In vivo cell-specific 'omics' and beyond

Recently, Fluorescence-Activated Cell Sorting (FACS) protocols have been applied to zebrafish embryos and now allows for isolation of fluorescently tagged cells from early zebrafish embryos to juvenile fish.²⁸ Interestingly, even discrete cell populations such as lateral line cells could be purified by FACS and were suitable for subsequent RNA extraction. Up to now, only proof-of-concept microarray assays were performed to validate this method. Recently, we purified neural stem cells (NSCs) from early zebrafish embryos undergoing neurogenesis.²⁹ Using the *gfap:GFP* transgenic line in which NSCs are fluorescent,³⁰ we successfully purified this specific cell population from whole embryos. Then, by comparing transcriptomes of NSCs purified from wild type embryos or from embryos in which specific morpholinos were injected, we were able to identify key genes that are more likely to be involved in a specific phenotype (e.g. spinal cord neurogenesis).³¹ Although we focused on *gfap:GFP*+ cells in this study, we also successfully purified other

neuronal cell populations following our FACS procedure (Fig. 2). Indeed, we also isolated nestin-positive neural progenitors from *nestin:GFP* transgenic one-day old embryos³⁰ (Fig. 2B) and post-mitotic neurons using the pan-neuronal transgenic line *elavl3:GFP*¹⁴ (Fig. 2C). Surprisingly, although the GFAP+ population was easily distinguishable from the negative pool of cells, it was not as trivial to discriminate nestin+ and *elavl3*+ cell populations (Fig. 2 compare green curves in A with B-C). However, when comparing with a GFP-negative control batch of cells (in purple in Fig. 2), we were able to set up scales that allow for a specific and selective sorting of GFP+ cells. This observation brings the fact that although this FACS-purification is a quite standardized technique, the sorting thresholds should be adapted for the desired application. In our work,²⁹ we wanted to sequence the RNA from these specific cell populations and therefore decided to be more stringent on the sorting threshold in order to avoid any false positive cells. On the contrary, if the aim of the experiment is to culture the purified cells, one could broaden these scales so as to obtain a higher chance of getting a growing cell culture that could further be screened down for GFP fluorescence.

To put in a nutshell, the strength of this technique is that it conserves the integrity of the embryonic *in vivo* context but *in fine* we only focused on our cells of interest. As a result, zebrafish embryos can be considered as *in vivo* test tubes in which cells of interest are growing in their intact endogenous *in vivo* environment rather than

Figure 2. FACS profiles of different neural transgenic lines. After cell-dissociation from 24-hours embryos, we were able to discriminate the GFP-positive population of cells from *gfap:GFP* (A), *elavl3:GFP* (B) and *nestin:GFP* (C) embryos. For each FACS experiment, a batch of non-GFP cells (WT) were used as a negative control and allowed to identify the non-overlapping GFP-specific population. Of note is that the number of cells to be sorted (y-axis) is different between lines depending of the percentage of cells expressing GFP.

in a petri dish. This strategy is opening new perspectives in deciphering the underlying endogenous molecular mechanisms of developmental phenotypes or teratogenic effects of toxic compounds.³²

Although we processed the purified cells for further transcriptomic analysis (RNA sequencing followed by differential gene expression analysis), this method can be used to unravel further omics studies. Indeed, immunoprecipitation (IP) and co-IP protocols have been adapted for zebrafish extracts,³³ thus cell-specific protein-protein interactions can now be analyzed *in vivo* in a cell-specific fashion. Moreover, chromatin-IP (ChIP) methods are also available from zebrafish extracts.³⁴ Combined with the latest Next Generation Sequencing techniques, it is possible to draw a map of the genomic binding sites of a protein of interest (ChIPseq) or to have access to the genome-wide methylation landscape (methylCap-seq³⁵) in a particular cell population that have been purified from *in vivo* embryos. Ultimately, using specific mass-spectrometry and other proteomics methods adapted to zebrafish,^{36,37} it expands endlessly the panel of genomic, transcriptomic and proteomic analysis that can now be performed in a cell-specific manner *in vivo*.

À-la-carte FISH'n CRISPs

Over the past few years, we observed an explosion of mutagenesis resources, especially with the development of the Clustered regularly interspaced short palindromic repeats (CRISPR) and CRISPR associated

endonuclease 9 (Cas9) that opened previously inconceivable possibilities for genome editing.³⁸ As a result, the number of gene function analysis by CRISPR-induced loss-of-function increased massively.³⁹ In these studies, random indels are generated at the locus of interest and a frameshifting mutation that encodes for a premature stop-codon is selected in the next generations. Moreover, rather than roughly disrupting the reading frame of the coding sequence, more subtle point mutations, mimicking human variants, have been precisely introduced within orthologous genes.⁴⁰ Similarly, larger fragments such as GFP or GAL4 coding sequences have been successfully knocked-in at a specific recombination site.^{41,42} Lastly, CRISPR interference (CRISPRi) gene knockdown can also be achieved using a catalytically inactive version of the Cas9 nuclease.⁴³ Likewise, the fusion of this inactive Cas9 with a transcription activator such as VP16 allows can be targeted to the regulatory regions of a gene of interest to promotes its endogenous overexpression (CRISPR-on).⁴⁴

Beyond the stripes: Translational neurosciences

The high percentage of orthology between human and zebrafish genes advanced scientists in their aim to mimic human disease conditions, in particular central nervous system dysfunctions.⁴⁵ Interestingly, thanks to the many advantages zebrafish depict and with the ability to now access cell-specific molecular mechanisms, the use of zebrafish for translational

Figure 3. Zebrafish as a model for translational neurosciences. After the identification of a gene or a mutation of interest (GOI/MOI) from genetic studies, this specific genetic condition can be modeled using CRISPR-derived genome editing tools. In about 9 months, an homozygous line can be raised and a cell-specific analysis can be performed. In the meantime, zebrafish can also be convenient for high-throughput drug screen seeking for a molecule rescuing a specific phenotype.

neurosciences is even more promising. In fact, complex neurological disorders such as epilepsy,⁴⁶ autism spectrum disorders,⁴⁷ schizophrenia⁴⁸ as well as motor neuron syndromes like amyotrophic lateral sclerosis, spinal muscular atrophy or hereditary spastic paraplegia⁴⁹ are being modeled in zebrafish and will undoubtedly shed light on up-to-now unknown mechanisms for these human diseases. Indeed, after genetic identification of specific human disease-causing-gene variants, this specific genetic condition can be mimicked in zebrafish using CRISPR-derived tools (Fig. 3). In less than a year, one can initiate *in vivo* cell-specific functional genomic studies on fish harboring the homozygous mutation of interest. As soon as a molecular, cellular and/or developmental phenotype is identified, one can start high-throughput drug screens in order to identify key molecules that can further be investigated for their therapeutic effects (Fig. 3).⁵⁰

In sum, with these new molecular tools in hands combined with the ease and handiness of zebrafish embryo manipulation, it has now become possible to generate à-la-carte genetic modifications *in vivo*. Moreover, by *in vivo* FACS purification, we are able to dissect cell-specific molecular mechanisms involved in custom genome-edited conditions.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

References

- [1] Kimmel CB, Ballard WW, Kimmel SR, Ullmann B, Schilling TF. Stages of embryonic development of the zebrafish. *Dev Dyn* 1995; 203:253-310; PMID:8589427; <http://dx.doi.org/10.1002/aja.1002030302>
- [2] Samarut E, Fraher D, Laudet V, Gibert Y. ZebRA: An overview of retinoic acid signaling during zebrafish development. *Biochim Biophys Acta* 2015; 1849:73-83; PMID:24928143; <http://dx.doi.org/10.1016/j.bbagr.2014.05.030>
- [3] Briggs JP. The zebrafish: a new model organism for integrative physiology. *Am J Physiol Regul Integr Comp Physiol* 2002; 282:R3-9; PMID:11742817; <http://dx.doi.org/10.1152/ajpregu.00589.2001>
- [4] Lohr H, Hammerschmidt M. Zebrafish in endocrine systems: recent advances and implications for human disease. *Annu Rev Physiol* 2011; 73:183-211; PMID:21314433; <http://dx.doi.org/10.1146/annurev-physiol-012110-142320>
- [5] Parichy DM. Advancing biology through a deeper understanding of zebrafish ecology and evolution. *Elife* 2015; 4; <http://dx.doi.org/10.7554/eLife.05635>
- [6] McCluskey BM, Postlethwait JH. Phylogeny of zebrafish, a 'model species,' within Danio, a 'model genus.' *Mol Biol Evol* 2015; 32:635-52; PMID:25415969; <http://dx.doi.org/10.1093/molbev/msu325>
- [7] D'Costa A, Shepherd IT. Zebrafish development and genetics: introducing undergraduates to developmental biology and genetics in a large introductory laboratory class. *Zebrafish* 2009; 6:169-77; PMID:19537943; <http://dx.doi.org/10.1089/zeb.2008.0562>
- [8] Schmidt R, Strahle U, Scholpp S. Neurogenesis in zebrafish - from embryo to adult. *Neural Dev* 2013; 8:3; PMID:23433260; <http://dx.doi.org/10.1186/1749-8104-8-3>
- [9] Blader P, Strahle U. Zebrafish developmental genetics and central nervous system development. *Hum Mol Genet* 2000; 9:945-51; PMID:10767318; <http://dx.doi.org/10.1093/hmg/9.6.945>
- [10] Kimmel CB. Patterning the brain of the zebrafish embryo. *Annu Rev Neurosci* 1993; 16:707-32; PMID:8460906; <http://dx.doi.org/10.1146/annurev.ne.16.030193.003423>
- [11] 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; 55:1-16; PMID:25109898; <http://dx.doi.org/10.1111/cga.12079>
- [12] Parng C, Roy NM, Ton C, Lin Y, McGrath P. Neurotoxicity assessment using zebrafish. *J Pharmacol Toxicol Methods* 2007; 55:103-12; PMID:16769228; <http://dx.doi.org/10.1016/j.vascn.2006.04.004>
- [13] Alexandre P, Reugels AM, Barker D, Blanc E, Clarke JD. Neurons derive from the more apical daughter in asymmetric divisions in the zebrafish neural tube. *Nat Neurosci* 2010; 13:673-9; PMID:20453852; <http://dx.doi.org/10.1038/nn.2547>
- [14] Park HC, Kim CH, Bae YK, Yeo SY, Kim SH, Hong SK, Shin J, Yoo KW, Hibi M, Hirano T, et al. Analysis of upstream elements in the HuC promoter leads to the establishment of transgenic zebrafish with fluorescent neurons. *Dev Biol* 2000; 227:279-93; PMID:11071755; <http://dx.doi.org/10.1006/dbio.2000.9898>
- [15] Nakano T, Wöndrem M, Zappavigna V, Goldman SA. Identification of a conserved 125 base-pair Hb9 enhancer that specifies gene expression to spinal motor neurons. *Dev Biol* 2005; 283:474-85; PMID:15913596; <http://dx.doi.org/10.1016/j.ydbio.2005.04.017>
- [16] Suster ML, Kania A, Liao M, Asakawa K, Charron F, Kawakami K, Drapeau P. A novel conserved *evx1* enhancer links spinal interneuron morphology and cis-regulation from fish to mammals. *Dev Biol* 2009; 325:422-33; PMID:18992237; <http://dx.doi.org/10.1016/j.ydbio.2008.10.004>
- [17] Blader P, Plessy C, Strahle U. Multiple regulatory elements with spatially and temporally distinct activities

- control neurogenin1 expression in primary neurons of the zebrafish embryo. *Mech Dev* 2003; 120:211-8; PMID:12559493; [http://dx.doi.org/10.1016/S0925-4773\(02\)00413-6](http://dx.doi.org/10.1016/S0925-4773(02)00413-6)
- [18] Shin J, Park HC, Topczewska JM, Mawdsley DJ, Appel B. Neural cell fate analysis in zebrafish using olig2 BAC transgenics. *Methods Cell Sci* 2003; 25:7-14; PMID:14739582; <http://dx.doi.org/10.1023/B:MICS.0000006847.09037.3a>
- [19] Halpern ME, Rhee J, Goll MG, Akitake CM, Parsons M, Leach SD. Gal4/UAS transgenic tools and their application to zebrafish. *Zebrafish* 2008; 5:97-110; PMID:18554173; <http://dx.doi.org/10.1089/zeb.2008.0530>
- [20] Kettleborough RN, Busch-Nentwich EM, Harvey SA, Dooley CM, de Bruijn E, van Eeden F, Sealy I, White RJ, Herd C, Nijman IJ, et al. A systematic genome-wide analysis of zebrafish protein-coding gene function. *Nature* 2013; 496:494-7; PMID:23594742; <http://dx.doi.org/10.1038/nature11992>
- [21] Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, Collins JE, Humphray S, McLaren K, Matthews L, et al. The zebrafish reference genome sequence and its relationship to the human genome. *Nature* 2013; 496:498-503; PMID:23594743; <http://dx.doi.org/10.1038/nature12111>
- [22] Schier AF. Genomics: Zebrafish earns its stripes. *Nature* 2013; 496:443-4; PMID:23594741; <http://dx.doi.org/10.1038/nature12094>
- [23] Qian X, Ba Y, Zhuang Q, Zhong G. RNA-Seq technology and its application in fish transcriptomics. *OMICS* 2014; 18:98-110; PMID:24380445; <http://dx.doi.org/10.1089/omi.2013.0110>
- [24] Samarut E, Gaudin C, Hughes S, Gillet B, de Bernard S, Jouve PE, Buffat L, Allot A, Lecompte O, Berekelya L, et al. Retinoic acid receptor subtype-specific transcriptotypes in the early zebrafish embryo. *Mol Endocrinol* 2014; 28:260-72; PMID:24422634; <http://dx.doi.org/10.1210/me.2013-1358>
- [25] Wan Y, Zhang Q, Zhang Z, Song B, Wang X, Zhang Y, Jia Q, Cheng T, Zhu X, Leung AY, et al. Transcriptome analysis reveals a ribosome constituents disorder involved in the RPL5 downregulated zebrafish model of Diamond-Blackfan anemia. *BMC Med Genomics* 2016; 9:13; PMID:26961822; <http://dx.doi.org/10.1186/s12920-016-0174-9>
- [26] Sleep E, Boue S, Jopling C, Raya M, Raya A, Izpisua Belmonte JC. Transcriptomics approach to investigate zebrafish heart regeneration. *J Cardiovasc Med (Hagerstown)* 2010; 11:369-80; PMID:20179605; <http://dx.doi.org/10.2459/JCM.0b013e3283375900>
- [27] Cox JA, Zhang B, Pope HM, Voigt MM. Transcriptome Analysis of Chemically-Induced Sensory Neuron Ablation in Zebrafish. *PLoS One* 2016; 11:e0148726; PMID:26863206; <http://dx.doi.org/10.1371/journal.pone.0148726>
- [28] Manoli M, Driever W. Fluorescence-activated cell sorting (FACS) of fluorescently tagged cells from zebrafish larvae for RNA isolation. *Cold Spring Harb Protoc* 2012; 2012; PMID:22854565; <http://dx.doi.org/10.1101/pdb.prot069633>
- [29] Samarut E, Bekri A, Drapeau P. Transcriptomic Analysis of Purified Embryonic Neural Stem Cells from Zebrafish Embryos Reveals Signaling Pathways Involved in Glycine-Dependent Neurogenesis. *Front Mol Neurosci* 2016; 9:22; PMID:27065799; <http://dx.doi.org/10.3389/fnmol.2016.00022>
- [30] Lam CS, Marz M, Strahle U. gfap and nestin reporter lines reveal characteristics of neural progenitors in the adult zebrafish brain. *Dev Dyn* 2009; 238:475-86; PMID:19161226; <http://dx.doi.org/10.1002/dvdy.21853>
- [31] McDermid JR, Liao M, Drapeau P. Glycine receptors regulate interneuron differentiation during spinal network development. *Proc Natl Acad Sci U S A* 2006; 103:9679-84; PMID:16763051; <http://dx.doi.org/10.1073/pnas.0504871103>
- [32] Santangeli S, Maradonna F, Gioacchini G, Cobellis G, Piccinetti CC, Dalla Valle L, Carnevali O. BPA-Induced Deregulation Of Epigenetic Patterns: Effects On Female Zebrafish Reproduction. *Sci Rep* 2016; 6:21982; PMID:26911650; <http://dx.doi.org/10.1038/srep21982>
- [33] Harrison MR, Georgiou AS, Spaink HP, Cunliffe VT. The epigenetic regulator Histone Deacetylase 1 promotes transcription of a core neurogenic programme in zebrafish embryos. *BMC Genomics* 2011; 12:24; PMID:21226904; <http://dx.doi.org/10.1186/1471-2164-12-24>
- [34] Lindeman LC, Vogt-Kielland LT, Alestrom P, Collas P. Fish'n ChIPs: chromatin immunoprecipitation in the zebrafish embryo. *Methods Mol Biol* 2009; 567:75-86; PMID:19588086; http://dx.doi.org/10.1007/978-1-60327-414-2_5
- [35] Bogdanovic O, Fernandez-Minan A, Tena JJ, de la Calle-Mustienes E, Gomez-Skarmeta JL. The developmental epigenomics toolbox: ChIP-seq and MethylCap-seq profiling of early zebrafish embryos. *Methods* 2013; 62:207-15; PMID:23624103; <http://dx.doi.org/10.1016/j.ymeth.2013.04.011>
- [36] Baral R, Ngounou Wetie AG, Darie CC, Wallace KN. Mass spectrometry for proteomics-based investigation using the zebrafish vertebrate model system. *Adv Exp Med Biol* 2014; 806:331-40; PMID:24952190; http://dx.doi.org/10.1007/978-3-319-06068-2_15
- [37] Link V, Shevchenko A, Heisenberg CP. Proteomics of early zebrafish embryos. *BMC Dev Biol* 2006; 6:1; PMID:16412219; <http://dx.doi.org/10.1186/1471-213X-6-1>
- [38] Hwang WY, Fu Y, Reyon D, Maeder ML, Tsai SQ, Sander JD, Peterson RT, Yeh JR, Joung JK. Efficient genome editing in zebrafish using a CRISPR-Cas system. *Nature Biotechnology* 2013; 31:227-9; PMID:23360964; <http://dx.doi.org/10.1038/nbt.2501>
- [39] Varshney GK, Burgess SM. Mutagenesis and phenotyping resources in zebrafish for studying development and human disease. *Brief Funct Genomics* 2014; 13:82-94; PMID:24162064; <http://dx.doi.org/10.1093/bfpg/elt042>

- [40] Armstrong GA, Liao M, You Z, Lissouba A, Chen BE, Drapeau P. Homology Directed Knockin of Point Mutations in the Zebrafish *tardbp* and *fus* Genes in ALS Using the CRISPR/Cas9 System. *PLoS One* 2016; 11:e0150188; PMID:26930076; <http://dx.doi.org/10.1371/journal.pone.0150188>
- [41] Auer TO, Duroure K, Concordet JP, Del Bene F. CRISPR/Cas9-mediated conversion of eGFP- into Gal4-transgenic lines in zebrafish. *Nat Protoc* 2014; 9:2823-40; PMID:25393779; <http://dx.doi.org/10.1038/nprot.2014.187>
- [42] Auer TO, Duroure K, De Cian A, Concordet JP, Del Bene F. Highly efficient CRISPR/Cas9-mediated knock-in in zebrafish by homology-independent DNA repair. *Genome Res* 2014; 24:142-53; PMID:24179142; <http://dx.doi.org/10.1101/gr.161638.113>
- [43] Larson MH, Gilbert LA, Wang X, Lim WA, Weissman JS, Qi LS. CRISPR interference (CRISPRi) for sequence-specific control of gene expression. *Nat Protoc* 2013; 8:2180-96; PMID:24136345; <http://dx.doi.org/10.1038/nprot.2013.132>
- [44] Cheng AW, Wang H, Yang H, Shi L, Katz Y, Theunissen TW, Rangarajan S, Shivalila CS, Dadon DB, Jaenisch R. Multiplexed activation of endogenous genes by CRISPR-on, an RNA-guided transcriptional activator system. *Cell Res* 2013; 23:1163-71; PMID:23979020; <http://dx.doi.org/10.1038/cr.2013.122>
- [45] Stewart AM, Braubach O, Spitsbergen J, Gerlai R, Kalueff AV. Zebrafish models for translational neuroscience research: from tank to bedside. *Trends Neurosci* 2014; 37:264-78; PMID:24726051; <http://dx.doi.org/10.1016/j.tins.2014.02.011>
- [46] Hortopan GA, Dinday MT, Baraban SC. Zebrafish as a model for studying genetic aspects of epilepsy. *Dis Model Mech* 2010; 3:144-8; PMID:20212082; <http://dx.doi.org/10.1242/dmm.002139>
- [47] Stewart AM, Nguyen M, Wong K, Poudel MK, Kalueff AV. Developing zebrafish models of autism spectrum disorder (ASD). *Prog Neuropsychopharmacol Biol Psychiatry* 2014; 50:27-36; PMID:24315837; <http://dx.doi.org/10.1016/j.pnpbp.2013.11.014>
- [48] Morris JA. Zebrafish: a model system to examine the neurodevelopmental basis of schizophrenia. 2009; 179:97-106; PMID:20302822
- [49] Patten SA, Armstrong GA, Lissouba A, Kabashi E, Parker JA, Drapeau P. Fishing for causes and cures of motor neuron disorders. *Dis Model Mech* 2014; 7:799-809; PMID:24973750; <http://dx.doi.org/10.1242/dmm.015719>
- [50] Delvecchio C, Tiefenbach J, Krause HM. The zebrafish: a powerful platform for in vivo, HTS drug discovery. *Assay Drug Dev Technol* 2011; 9:354-61; PMID:21309713; <http://dx.doi.org/10.1089/adt.2010.0346>