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Meeting report

The rising zebrafish research in China: Meeting report of the 3rd Chinese Zebrafish Principal Investigator Meeting & the Inaugural Meeting of China Zebrafish Society

The zebrafish has become a very important animal model, not only for developmental biology and genetics, but also for disease modeling and drug discovery. Since the year of 2000, zebrafish research has been rapidly growing in China, and the laboratories using zebrafish as the main subject have been increasing dramatically (Xie et al., 2015). For example, the number of participants who attended the Chinese Zebrafish Principle Investigator (PI) Meeting series is 80 in 2012, 150 in 2014, and over 200 this year.

With the rapid increase of zebrafish researchers in China, the Chinese zebrafish community decided to hold the National Zebrafish Research Conference of China (NZRC) & the Chinese Zebrafish Principle Investigator Meeting (CZPM) every other year, with NZRC in the odd-numbered years and CZPM in the even-numbered years. Meanwhile, there is an urgent need to set up an official organization for the Chinese zebrafish community. On Sep 23–26, 2016, the 3rd CZPM & the Inaugural Meeting of China Zebrafish Society (CZFS) were held successfully in Wuhan, a beautiful city in central China and the location of China Zebrafish Resource Center (http://zfish.cn). More than 200 zebrafish PIs participated in this combined meeting. There are 21 plenary talks and 44 concurrent session talks, and the topics cover from early development, organogenesis, hematopoiesis, neurogenesis, to toxicology and disease models. During the two and half day meeting, the conference attendees discussed both scientific issues in the field and other related issues such as grant application and zebrafish resource collection.

On the morning of Sep 24, Anming Meng (Tsinghua University) gave an opening keynote lecture in which he introduced the history of zebrafish research in China, the rapid increase of zebrafish laboratory number and zebrafish-related publications (Fig. 1). In the year of 2015, according to the statistical analysis of the data from the Core Collection Database (v5.20) of Web of Science™ (Liu et al., 2016), the total number of zebrafish publications in China, for the first time, reached the half of that in USA and many of them were published in high-profile journals such as *Immunity*, *Neuron*, *Developmental Cell*, and *Development*. The research area spans widely from developmental biology, cell biology, biochemistry and molecular biology, new technology to toxicology and environmental science. He also suggested that zebrafish researchers in China should focus on fundamental questions with unique feature and try to fill the gap between basic science and clinical medicine with the help of new technology. Finally he emphasized that the newly established CZFS should make a team work. In the following talk, Feng Liu (Institute of Zoology, Chinese Academy of Sciences) briefly introduced the recently completed project by "Zebrafish All Genes KO Consortium for Chromosome 1 (ZAKOC)", which is a great example for CZFS as a team to accomplish an important mission in such a short period. The initial phase of this project aims to knockout a total of 1333 genes in Chromosome 1. We believe this is the first trial to systematically target all genes in an entire chromosome in the genome of a complicated vertebrate species.

As the first invited plenary speaker, Hong Zhang (Institute of Biophysics, Chinese Academy of Sciences) presented his recent work on autophagy regulation. Using *Caenorhabditis elegans* as a model, his group identified a new autophagy gene, *epg5*, and loss of *epg5* causes accumulation of non-degradative autophagic vacuoles (Wang et al., 2016). Interestingly, EPG5 deficiency in mice showed neurological defects, which mimics Vici syndrome in humans (Zhao et al., 2013). It is noteworthy that the 2016 Nobel Prize in Physiology or Medicine was awarded to Yoshinori Ohsumi "*for his discoveries of mechanisms for autophagy*". Next, Enkui Duan (Institute of Zoology, Chinese Academy of Sciences) talked about a new type of tRNA-derived small RNA, tsRNAs, isolated from sperm and serum in mice, and demonstrated that tsRNAs in sperm carry important epigenetic information in controlling intergenerational transmission of acquired metabolic disorders such as diabetes (Wang et al., 2016).

In the following talks, over 60 zebrafish PIs presented their recent data in a broad range of topics from development, disease models to toxicology. Next, we will briefly summarize their talks and highlight the exciting new findings during the meeting.

1. Early development and reproduction

Zebrafish embryos have been utilized to study early development widely and many speakers shared their recent findings in this session. Shunji Jia (Tsinghua University) showed that the zebrafish/human phosphatidylinositol transfer protein Sec14l3/SEC14L2 acts as GTPase proteins to mediate Wnt/Ca^{2+} signaling and this signal cascade is important for zebrafish embryonic convergent and extension. Yuhua Sun (Institute of Hydrobiology, Chinese Academy of Sciences) discovered that zebrafish *mga* is involved in the development of neural crest cells through indirect regulation of BMP signal. Yun Liu (Sun Yat-Sen University) provided genetic evidence that miR-430 is required for the establishment of embryonic body plan by clearance of the maternal transcripts and activation of zygotic genome during early embryonic development. Jianfeng Zhou (Ocean University of China) presented that GPx4 (glutathione peroxidase 4) inhibits Wnt/β-catenin signaling and regulates dorsal organizer formation in zebrafish embryos.

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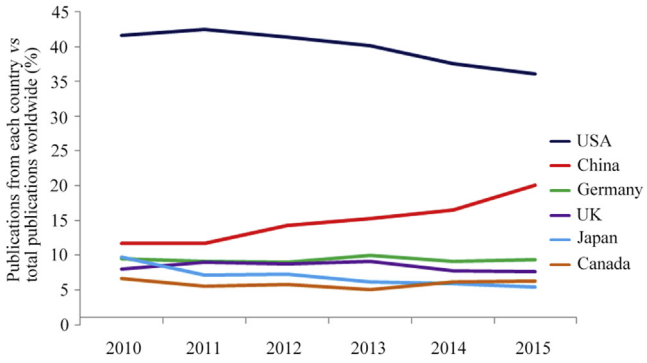

Fig. 1. Zebrafish-related publications from China and other major zebrafish research countries in 2010–2015.

Wuhan Xiao (Institute of Hydrobiology, Chinese Academy of Sciences) reported data on an androgen receptor zebrafish mutant and discovered that androgen receptor is required for spermatogenesis and ovarian function in zebrafish, which provides new insights for sex determination and might be applicable in aquaculture. Using a newly generated *gtsf1*$^{-/-}$ mutant by TALEN, Jihua Yao (Fudan University) characterized the mutant with the phenotype of male sterility, suggesting that *gtsf1* may participate in the process of spermatocytogenesis. Haipei Tang (Sun Yat-sen University) presented that LH (luteotropic hormone) regulates zebrafish ovulation through controlling the synthesis processes of prostaglandin and the expression of prostaglandin receptors.

2. Organogenesis

Jinrong Peng (Zhejiang University) talked about the role of *hhex* (hematopoietically expressed homeobox) in the development of digestive system. Loss of *hhex* not only affects development of the liver and pancreas, but also leads to an intrahepatic intestine in the *hhex*$^{-/-}$ liver. *Hhex* and a number of transcription factors form a genetic network to orchestrate the organogenesis of the hepatopancreatic ductal (HPD) system.

Cilia are conserved and broadly distributed organelle in all vertebrate cells and have been shown to play essential roles in organogenesis. Ciliopathy can cause multiple genetic diseases including polycystic kidney disease (PKD). Using CRISPR/Cas9 system, Ying Cao (Tongji University) discovered that apical ptdins P2 is required for ciliogenesis in zebrafish (Xu et al., 2016c). Moreover, through generation of maternal-zygotic mutants of *talpid3* and *kif7*, Jin Ben (Kunming University of Science and Technology) presented data showing that the synergistic effect between Kif7 and Talpid3 is important for ciliogenesis through regulation of Hedgehog signaling.

Oxygen is essential for all animals. Cunming Duan (University of Michigan) discussed oxygen-dependent and independent actions of hypoxia-inducible factors (HIFs). Interestingly, a truncated Hif3α lacking the oxygen-dependent degradation domain can bind to β-catenin and induce its degradation. Therefore, he identified a dual role of Hif3α in response to oxygen and negative regulation of Wnt/β-catenin signaling (Zhang et al., 2016). In addition, overexpression of Hif3α2 can lead to left-right asymmetry defects.

Zhonghua Zhao (Shanxi University) presented a null allele of *grk2* (G protein-coupled receptor kinase 2) mutant in zebrafish by ZFN, which shows abnormal formation of swim bladder at 4 dpf, and he demonstrated that GRK2 plays an essential role in transducing the Hedgehog signaling. By establishing a *stat3* mutant with TALEN, Jie Mei (Huazhong Agricultural University) demonstrated that *stat3* mutation can cause spine malformation and immune disorder in zebrafish.

The study of skeletal muscle growth is predominant in animal husbandry. Shuming Zou (Shanghai Ocean University) reported that *gcfst2* overexpression enhanced muscle growth in blunt snout bream, which might be useful for promoting directional breeding in aquaculture.

3. Heart development and tissue regeneration

Jun Zhu (Shanghai Jiao Tong University) presented their new finding on the role of Gata5 SUMOylation, which is indispensable for cardiac development in zebrafish. They showed that GATA5 can be modified by SUMO. While this modification is dispensable for its nuclear localization, it is essential for its transcriptional activity. They further identified that K360 is the major site for SUMOylation of GATA5. In addition, Wuzhou Yuan (Hunan Normal University) talked about a new TSMR1-GFP transgenic zebrafish for lineage tracing analysis in heart development. Rongjia Zhou (Wuhan University) reported that zebrafish pluripotent stem cells can be induced to cardiomyocytes by certain factors. Electrophysiological characterization implicated that the induced cardiomyocytes functionally responded to physical stimulation (Xiao et al., 2016).

Heart regeneration occurs by activating dedifferentiation and proliferation of resident cardiomyoctyes (CMs) and zebrafish has become a powerful model for adult organ regeneration. However, the underlying mechanisms remain elusive. Tao Zhong (Fudan University) showed that a series of negative regulators of Wnt signaling are induced after cardiac injury. Manipulation of Wnt signaling using heat-shock inducible tools can affect heart regeneration through altering nuclear translocation of β-catenin. Jing-Wei Xiong (Peking University) continued his talk on heart regeneration and discussed their findings in this process. His group identified that H_2O_2 promotes heart regeneration through a depression mechanism involving Dusp6 (Han et al., 2014). Furthermore, Brg1 negatively regulates cell cycle inhibitors including Cdkn1a and Cdkn1c in the myocardium to promote CM proliferation after heart injury. They also developed a novel nanoparticle to specifically deliver siRNA to CM (Diao et al., 2015).

Finally, Dong Liu (Peking University) presented their recent data on the signaling and cellular basis of zebrafish neuromast hair cell regeneration. Using the zebrafish lateral line system (the inner ear on the skin) as a model, they demonstrated that the cell cycle state of supporting cells can determine the mode of hair cell regeneration and that these supporting cells can also be converted into new hair cells during regeneration.

4. Hematopoiesis, innate immunity and vessel repair

The first lecture on hematopoiesis was given by Feng Liu (Institute of Zoology, Chinese Academy of Sciences) talking about a recent finding in development of hematopoietic stem cells (HSCs). In contrast to the well-known role of Notch signaling in enhancing HSC emergence, he found that Notch is dynamically expressed in the process of HSC development, i. e., Notch is required for artery establishment as well as specification of hemogenic endothelium, but plays an inhibitory role for HSC emergence. They demonstrated that a G protein coupled receptor, GPR183, regulates the stability of Notch receptor through β-Arrestin-Nedd4 complex (Zhang et al., 2015). Anskar Leung (The University of Hong Kong) followed with the regulation in HSC development and described a novel role of Arl4a in definitive hematopoiesis. Knockdown or knockout of *arl4aa* in zebrafish leads to defects in HSCs and this is due to a specific Golgi defect in hemogenic endothelium while not affecting Golgi complex integrity in other tissues. They further showed that overexpression of Notch intracellular domain (NICD) can rescue *arl4aa* morphants, suggesting that Notch signaling is downstream of Arl4a. Xin Li (Huazhong University of Science and Technology) told us about the super-enhancers (SEs) in zebrafish adult kidney

which is adult hematopoietic organ in teleost, and compared the SEs between kidney and liver in adult zebrafish.

Liangbiao Chen' group (Shanghai Ocean University) talked about their finding in the Antarctic icefish—a unique fish clade that evolve in extremely cold environment. His group showed that evolutionary suppression of erythropoiesis by low temperature is through miRNA biogenesis regulated by TGFβ and P53. By comparing the expression profiling between Tilapia and zebrafish, they showed that cold-induced apoptosis in the gills is mediated by FoxO pathway (Hu et al., 2016).

Innate immunity acts as the first defense line in human body but the regulation mechanism of innate immunity is still unclear. Qinghua Zhang (Shanghai Ocean University) described a *notch1a* CRISPR/Cas9 mutant with somite formation defects, affecting immune response while defensing *Vibrio parahaemolyticus*, and her group found that *notch1a* might mediate innate immune response. Ce Ji (Shanghai Ocean University) used RNA-seq technology to study the mechanism of the innate immune response in zebrafish. Qiaoqing Xu (Yangtze University) showed that IRF4a/b displayed a distinct expression pattern *in vivo* and *in vitro* and IRF4 paralogues might play different roles in immune system.

The origin of tissue resident macrophages in mammals remains controversial. Jin Xu (The Hong Kong University of Science and Technology) presented that microglial (macrophages in the brain) are derived from both the rostra blood island (RBI) and the ventral wall of dorsal aorta (VDA) in zebrafish and their migration into the brain is mediated by lysophosphatidylcholine (LPC) secreted by apoptotic neurons (Xu et al., 2015). Moreover, neuron specific overexpression of Bcl2 can block apoptosis that will consequently inhibit microglial colonization (Xu et al., 2016a).

As the winner of '2016 Zeiss zebrafish research scholar award', Lingfei Luo (Southwest University) reported an unexpected function of macrophages in the repair of brain vascular rupture. Through time-lapse confocal imaging, his group elegantly showed that microglial can repair brain injury through extending filopodia and this process is dependent on chemokine-induced phosphatidylinositide 3-kinase and Rac1 activity (Hu et al., 2016). Wei Hu (Institute of Hydrobiology, Chinese Academy of Sciences) reported that SNb (secretoneurin) is a type of neuropeptides, which is required for development of central arteries (CtAs) in zebrafish. This finding implies that the encoding gene *sgII* may serve as a potential target for treatment of vessel-related diseases in the central nervous system.

5. Neuroscience

Jie He (Institute of Neuroscience, Chinese Academy of Sciences) talked about the origin of postembryonic stem cells in the retina. Using a cytobow-based approach for lineage tracing, he was able to show that these post-embryonic retinal stem cells are derived from their embryonic counterparts.

Habenula, a hub of neuromodulatory systems, is a control center of mood in humans. Using a series of wide-field imaging, visual stimulation, neural computation and network reconstruction, behavior monitoring, optogenetics, whole-cell recording and circuit dissection, Jiulin Du (Institute of Neuroscience, Chinese Academy of Sciences) elegantly showed that left habenula mediates light-preference behavior *via* receiving visual inputs. This finding may serve as a node for crosstalk between sensory and emotional systems (Yao et al., 2016).

6. Technologies and interdisciplinary research

All the known engineered endonucleases used for DNA editing are based on sequence preference including ZFN, TALEN, and CRISPR/Cas9. Qingshun Zhao (Nanjing University) developed an alternative novel tool for DNA editing without target sequence limitation, the structure-guided endonuclease (SGN) (Xu et al., 2016b). Importantly, his group has demonstrated the SGN's application in zebrafish embryos by cleaving transgenic reporter gene and endogenous genes albeit with low efficiency.

Enhancer trapping is often used to generate tissue- or cell-specific reporter lines; however, the specificity and efficiency of the available trapping vectors remain to be improved. Zongbin Cui (Institute of Hydrobiology, Chinese Academy of Sciences) developed an enhancer-trapping vector pTME that contains a minimal mouse metallothionein gene promoter upstream of EGFP reporter as well as a mutation-cassette. Therefore, the pTME can be used as an alternative tool for both enhancer trapping and mutagenesis in zebrafish (Liu et al., 2015). Dan Shen (Yangzhou University) reported a high efficient transposon in zebrafish which can also mediate gene transposition in mammals.

Yinmei Li (University of Science and Technology of China) presented an exciting application of optical manipulation of cells *in vivo* and recommended a powerful technique (optical tweezers) for basic and clinical sciences. Similarly, Zuxiang Liu (Institute of Biophysics, Chinese Academy of Sciences) reported the application of optogenetics in the study of visual function. Fangyi Chen (Southern University of Science & Technology) developed an instrument system, which is composed of a rotational platform, a digital microscope and an image processing software, to measure the vestibular ocular reflex of zebrafish larvae.

Desheng Pei (Institute of Green and Intelligent Technology, Chinese Academy of Sciences) talked about the characterization of organic compound (hair and skin) derived carbon quantum dots (CDs) and suggested that they can be used as long-term bioprobes in zebrafish studies, compared to citric acid derived CDs. Fan Yang (Hubei University of Chinese Medicine) introduced a high efficient-zebrafish embryo handling platform based on microfluidic tools and discussed potential applications of this "fish-on-a-chip" technology.

7. Disease models

The circadian clock modulates a diverse of fundamental processes as an endogenous time-keeping mechanism. Han Wang (Soochow University) demonstrated the role of the circadian clock in psychiatric diseases using zebrafish models. Using multiple behavior assays, he showed that zebrafish *per2* mutant fish display a clear depression phenotype, which is likely mediated by downregulation of glucocorticoid receptor (gr) signaling. Bing Hu (University of Science and Technology of China) reported that an *aanat2* mutant lacking endogenous melatonin displayed disrupted migrating rhythmic of neutrophils towards the injury in zebrafish and again emphasized the significant function of circadian clock. Li Yang (Peking University) reported that knocking down of zebrafish *micall2b* can mimic the phenotype of human attention deficit hyperactivity disorder (ADHD).

By generating the *bloc1s1* (biogenesis of lysosome-related organelles complex 1 subunit 1) mutant in zebrafish, Kaiyao Huang (Institute of Hydrobiology, Chinese Academy of Sciences) showed that loss of *bloc1s1* can disrupt the biogenesis of iridophores. Although it has been demonstrated in mice that BLOC1S1 is involved in autophagy, it is not the case in zebrafish. He further showed that lamellar body-like organelles were accumulated in the swim bladder in the mutant.

Zhan Yin (Institute of Hydrobiology, Chinese Academy of Sciences) showed that vitamin D/vitamin receptor (VDR) signaling regulates lipid metabolism in zebrafish visceral adipose tissues. Using the newly generated *cyp2r1* mutants by TALEN, he and his colleagues identified a number of target genes of the VDR signaling including *pgc-1*. They also showed the impaired mitochondrial biogenesis and oxidative metabolism in the mutant fish. Mingyu Li (Xiamen

University) talked about his recent work with zebrafish *gcgra* and *gcgrb* double knockout mutant to search for factors promoting pancreatic α cell proliferation. Small molecule screening showed that ErbB receptor family is required in this process and this has been confirmed in isolated pancreatic islet in mice (Li et al., 2015).

Retinal photoreceptor is the major cause of adult blindness. Mugen Liu (Huazhong University of Science and Technology) showed that the *rp2* mutation can cause photoreceptor cell death at later stages in zebrafish by disrupting the protein stability. Jing Tian (Northwest University) described that a new zebrafish mutant can partially mimic Cenani-Lenz syndactyly syndrome. It was further demonstrated that Wnt signaling is dysregulated which thereby affects Notch signaling in the kidney. By generating an erythrocyte-specific transgenic zebrafish with a deficiency of 118–144 site on *g6pd*, Li-Ping Shu (Guizhou Medical University) proposed a zebrafish disease model with G6PD deficiency, which may help to explore the underlying mechanism and to identify antimalaric drugs by high-throughput screening.

8. Toxicology

Zebrafish is considered as a great model for the assessment of developmental toxicity during drug screening and environmental pollution testing. Qingchun Zhou (Central China Normal University) introduced that oxindoles affects early development of zebrafish heart. Wu Dong (Inner Mongolia University for Nationalities) talked about the neurotoxicity of polybrominated diphenyl ethers in zebrafish embryos. Xiaojing Meng (Southern Medical University) and Yao Dang (Huazhong Agricultural University) reported that Indium and Prochloraz might impair zebrafish reproduction, respectively. Shan Zhong (Wuhan University) talked about the function of zebrafish Abcc4 protein during toxin extrusion process. Tingting Chai (Institute of Quality Standard and Testing Technology for Agro-Products of CAAS) investigated the toxicity of polychlorinated biphenyis pollution through zebrafish metabonomics analysis. Kechun Liu (Biology Institute of Shandong Academy of Sciences) introduced the platform of evaluation of chemical toxicity based on zebrafish model.

9. Closing remarks

The 3rd Chinese Zebrafish PI meeting (CZPM) & the Inaugural Meeting of China Zebrafish Society (CZFS) provides a great platform for zebrafish researchers in China to communicate the latest advances in science, and also facilitates potential collaboration in the future. By doing so, many PIs with similar research interest can easily make a team to endeavor big scientific questions, such as neuron circuitry, disease modeling, and drug screening. Therefore, we strongly believe that with the completion of this combined meeting, both the scientific quality and the impact of Chinese zebrafish research in the world will increase remarkably in the near future. As decided by the newly founded CZFS, the 5th NZRC will be held in Shanghai on Oct 13–16, 2017, and the 4th CZPM will be held in Wuhan in Oct 2018, and we look forward to seeing you all in the next meetings!

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