Utilization of Zebrafish as a Model System in Medical Research

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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...
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.

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