Zebrafish: Neurological Diseases

Subjects: Others

Contributor: Jennifer Freeman, Arjun Pitchai

Most neurodegenerative diseases are currently incurable, with large social and economic impacts. Recently, there has been renewed interest in investigating natural products in the modern drug discovery paradigm as novel, bioactive small molecules. Moreover, the discovery of potential therapies for neurological disorders is challenging and involves developing optimized animal models for drug screening. In contemporary biomedicine, the growing need to develop experimental models to obtain a detailed understanding of malady conditions and to portray pioneering treatments has resulted in the application of zebrafish to close the gap between in vitro and in vivo assays. Zebrafish in pharmacogenetics and neuropharmacology are rapidly becoming a widely used organism. Brain function, dysfunction, genetic, and pharmacological modulation considerations are enhanced by both larval and adult zebrafish. Bioassay-guided identification of natural products using zebrafish presents as an attractive strategy for generating new lead compounds. Here, we see evidence that the zebrafish's central nervous system is suitable for modeling human neurological disease and we review and evaluate natural product research using zebrafish as a vertebrate model platform to systematically identify bioactive natural products. Finally, we review recently developed zebrafish models of neurological disorders that have the potential to be applied in this field of research.

Keywords: Bioassay-guided purification ; Neurodegenerative model ; zebrafish

1. Introduction

The zebrafish is being progressively used to model neurodegenerative diseases and neurological disorders successfully , with promises to test potential treatments for diseases and disorders. The zebrafish CNS is similarly arranged to that of other vertebrates, and is traditionally separated into the hindbrain, midbrain, forebrain, ascending and descending spinal cord, cranial nerves, motor spinal cord, and sensory nerves. Zebrafish neuroanatomy has been examined and described in detail elsewhere during development, as well as in adults. The genome of the zebrafish is widely annotated. The evolutionary lineage of zebrafish (teleost-bonyfish) separated about 450 million years ago from the human lineage (tetrapod). Zebrafish pairs can produce large number of embryos that make it possible to achieve relatively high-throughput screening drug studies and behavioral testing with simple methods for modulating gene expression available. Many human-associated neurodegenerative disease proteins in zebrafish are homologous, highlighting potentially preserved molecular cellular functions that can be easily examined.

2. Zebrafish and Neurological Diseases

2.1 Zebrafish and Alzheimer’s Disease

The most common form of irreversible neurodegenerative disorder and dementia is Alzheimer’s disease (AD). Fifty million people were estimated to live with AD in 2018, and this figure is predicted to increase to 152 million by 2050. AD’s main clinical feature is progressive memory loss, motor and speech impairment, deception, depression, and aggressive behavior. There is significant neuronal damage in AD patients in numerous brain regions. This is usually caused by extracellular deposition of amyloid-beta peptide and tau protein aggregates called neurofibrillary tangles (NFTs). Several risk factors are identified or under investigation, including both genetic and environmental factors, as potential triggers of AD. AD may be classified as familial AD (FAD, at < 65 years of age) or sporadic AD (SAD, at > 65 years of age). Most of the knowledge of AD pathogenesis has been defined by studying FAD mutations. Some of the genetic targets are precursor protein amyloid-ß (*APP*) and presenilins (*PSEN1* and *PSEN2*) associated with increased FAD risk. The more common form of AD occurs sporadically (representing >90% of cases). Multi-faceted pathogenesis of SAD is associated with several risk factors such as old age and the presence of the apolipoprotein (*APOE*) gene ε4 allele and/or the recently identified genetic risk factor sortilin-related receptor (*SORL1)*. SORL1 is an APOE receptor with primary expression in neurons of the brain.

Zebrafish have human orthologous genes that play key roles in AD. The zebrafish genes *psen1* and *psen2* are human *PSEN1* and *PSEN2* orthologs, respectively, whereas the genes *appa* and *appb* are human *APP* co-orthologs. The zebrafish genome also contains orthologous genes for gamma-secretase's complex components, *PSENEN (psenen)*, *NCTN (ncstn)* , and *APH1b (aph1b)*. In addition, β-secretase orthologs (*BACE1* and *BACE2*) were also identified (*bace1* and *bace2*) in zebrafish. The zebrafish genome contains co-orthologs of the microtubule-associated tau protein (*MAPT*) gene, which encodes tau protein (*mapta*, and *maptb*). The human *APOE* and *SORL1* co-orthologs *apoea* and *apoeb* are also present in the zebrafish genome and *sorl1*, respectively.

2.2 Zebrafish and Parkinson's Disease

Dopaminergic neuron degeneration, as well as the presence of Lewy bodies called intracytoplasmic inclusions, are neuropathological lesions associated with Parkinson's disease (PD). Six genes associated with Parkinsonism have been identified, including *Parkin*, *DJ-1*, *PINK1*, *α-Synuclein*, *UCHL-1*, and *LRRK2* . Although predominantly a motion disorder, PD is a mixed group of neurological conditions that are not capable of producing or controlling movement and cognitive impairment. The human *PARK2* ortholog in zebrafish (*park2*) resides on chromosome 13, and encodes a protein of 458 amino acids (465 in humans). The *PINK1* zebrafish ortholog has 54% similarity, and an initial study reported a severe developmental phenotype in pink1 k/d zebrafish. The *PARK7* zebrafish ortholog encodes a protein of 189 amino acids with 83% human DJ-1 identity.

2.3 Zebrafish and Huntington's and Other Polyglutamine Diseases

Huntington's disease (HD) is a monogenic neurodegenerative disease that follows an autosomal dominant pattern of huntingtin gene mutant form (*HTT*) inheritance. The mutation encodes for an abnormal trinucleotide that leads to glutamine (CAG) expansion at the HTT protein amino terminal and arises in an extended polyglutamine tract of the Huntingtin protein. This causes cell death by gain of function mechanisms, like protein accumulation, mitochondrial dysfunction, and caspase activation. A decline in normal Huntingtin can also make a significant contribution to pathogenesis. To try to elucidate the loss as well as the gain of function mechanisms, zebrafish models are being used. The HD cDNA homology in zebrafish was isolated as the first step towards discovering the possible role of the HD gene in initial vertebrate development. This cDNA codes a predicted protein product of 3121 amino acids with a human HTT identity of 70%. Loss of developmental expression of 15/hd1 caused noticeable morphological abnormalities, including pericardial edema microcephalus and CNS necrosis. Zebrafish *htt* is also necessary for normal pharyngeal arch cartilage development.

2.4 Zebrafish and Amyotrophic Lateral Sclerosis (ALS)

Amyotrophic lateral sclerosis (ALS) is characterized by protein inclusions present in the affected neurons. These protein inclusions are linked to spinal cord motor neuron loss and downward motor tracts in the brain and spinal cord. Familial ALS is fairly rare, but a gene-encoding mutation of superoxide dismutase (*SOD1*) inherited in an autosomal dominant motif causes 20% of such cases. The mutations are usually prescribed by gain of function mechanisms. Over 150 mutations have been discovered in *SOD1*, including the point mutations G93R and G85R. Recent studies also indicate a role for *SOD1* in the sporadic form of ALS and propose a prion-like function of protein misfolding. Moreover, a few of the recently identified genes involved in ALS, such as *FUS* and *TARDBP*, also demonstrate a high tendency to act similar to prions in misfolding proteins.

A recent study used zebrafish to assess overexpression of *SOD1* by mRNA microinjection to study ALS etiology. In this study, vascular endothelial growth factor (*VEGF*) overexpression rescued the *SOD1*-expressing zebrafish axonopathy, while *VEGF* morpholino knockdown exacerbated the abnormalities. However, one of the limitations in working with ALS in vivo models is the lack of comprehensive methods to assess the presymptomatic course of the disease. The zebrafish provides advantages in the study of processes of early disease with rapid development and reach post-embryonic life about 3 days post fertilization (dpf), which is similar to neonatal mouse development.

2.5 Zebrafish and Schizophrenia

Schizophrenia is a severe neurodegenerative disorder with the etiology of hallucination, delusions, depression, agitated body movements, confused thoughts and snafu speech, anhedonia, lack of motivation, and speech problems. The defects of schizophrenia are caused by early development in the brain. About 1% of the world's population is affected by schizophrenia and is characterized by neuronal dysfunction, which results in deficiencies in various cognitive areas including visual and verbal memory, learning, and attention. Patients with schizophrenia, as well as with HD, have impaired preimpulse inhibition (PPI), a type of sensorimotor gaiting. PPI is a neurological event where the response following shocking stimulus is defeated by a weak prestimulus or prepulse and is conserved among vertebrates. The

sensorimotor zebrafish gating has been described in 6 dpf larvae for PPI testing. Twin studies have a projected heritage of around 81% for schizophrenia and an environmental impact of about 11% (factors such as diet, parenting, and exposure to toxins or teratogens). A large number of cases of schizophrenia are sporadic and appear in a family without a history of the disease. Many genes have been linked to schizophrenia susceptibility. Genes with a largely robust disease connection include dystrobrevin binding protein 1 (*DTNBP1*), neuregulin1, disrupted in schizophrenia1 (*DISC1*), kinesin family member 1 (*KIF1*), kinesin family member 17 (*KIF17*), *SH3*, multiple ankyrin repeat domains 3 (*SHANK3*), and *NOTCH4*. Candidate genes for schizophrenia may be vital in determining neuronal migration, neurogenesis, and cell fate .

Burgess and Granato developed an endophenotype of schizophrenia in zebrafish PPI. Exposure to apomorphine and ketamine influences zebrafish PPI, and therefore appears to be facilitated by similar neurotransmitters as in other animals. The same study also identified five novel mutant lines with abnormal PPI responses. One of the most intensively studied genes associated with schizophrenia is *DISC1*, and was first identified with a high incidence of depression, schizophrenia, and bipolar disorder in a Scottish pedigree. Furthermore, *disc1* studies in zebrafish have provided new information on this gene's function.

2.6 Zebrafish and Epilepsy

Epilepsy is a common neurological disease caused by unexpected seizures that can vary from a short attention interval to severe and prolonged seizures and muscle cramps. Epilepsy has a pathological mechanism that is poorly understood and is a complex brain disorder with many fundamental causes. Zebrafish have a multifaceted nervous system with elegant behaviors, and are prone to seizure. Adult zebrafish have a wide array of established behaviors that can be studied, making them especially suitable for model development. The pentylenetetrazole (PTZ)-induced zebrafish epileptic seizure has been used to study the mechanism of epilepsy. The affordability of both larval and adult zebrafish, which allows the ontogenesis to investigate a wider range of epilepsy-related phenomena, is also useful.

Several genetically altered zebrafish are now being used to study the behavior and brain function associated with epilepsy. Zebrafish (~5–7 dpf) are commonly placed in multiple wells and tracked using video tracking software, continuously recorded by a camera. Mutations in two family members, Potassium Voltage-Gated Channel Subfamily Q Member 2 (*KCNQ2*) and Potassium Voltage-Gated Channel Subfamily Q Member 3 (*KCNQ3*), have been correlated with inherited neonatal epilepsy, e.g., benign family neonatal convulsions. These genes are highly expressed in zebrafish, providing support for studies of epilepsy using this vertebrate model.

2.7 Zebrafish Bioassay-Guided Isolation of Natural Product Drug Discovery

Zebrafish was first suggested by Jones and Huffmann of the Oklahoma Research Foundation as a model for in vivo drug development in 1957, and soon thereafter zebrafish were first used to examine NP bioactivities. Zebrafish bioassay-guided identification of NPs in a number of neurological disorders can be an attractive approach for generating novel lead compounds (Figure 1). Over the past decade, zebrafish as a primary model for HTS in the scope of drug discovery for NPs for neurological disease has grown. Zebrafish model profits combined with robust chromatographic and spectroscopic methods are creating a path to discover and further develop HIT compounds from various plant extracts. Zebrafish has recently emerged as a strong model in a wide range of applications for rapid analysis of gene function and small molecular bioactivity. Zebrafish are well-suited to identify therapeutically potential bioactive NPs (Table 2). Zebrafish were first proposed over fifty years ago as an in vivo model for the discovery of small molecules of drugs. This preliminary study examined the utility of zebrafish embryos and larvae to screen both NPs and synthetic compounds. Zebrafish offers the opportunity of in vivo swift microgram-scale bioactivity evaluation of small molecules, an attractive feature combined with high-resolution fractionation technologies and analytical methods like UHPLC-TOF-MS and NMR microflow. A recent example is the bioassay-guided isolation of zebrafish with six spirostane glycosylated triterpene important for decoction and methanol extract anti-sizing activity from *Solanum torvum* aerial parts, which was discovered by Soura Challal and his colleagues. In addition, the recently identified flavonoid-*trans*-tephrostachin inhibitory of acetylcholinesterase has also been isolated from the leaves of Indian herb *Tephrosia purpurea* by a zebrafish bioassay.

2.8 Generation of a Neurodegenerative Model Using Amyloid-β42 (Aβ42) in the Adult Zebrafish Brain

The zebrafish bear extensive regenerative ability, and clinically important studies are aimed at understanding the mechanisms of zebrafish regeneration. Zebrafish are excellent tools because of their CNS regenerative capacity. Models of neurodegeneration in the adult zebrafish brain will be helpful to investigate the activation state of the neural stem/progenitor cells (NSPCs) and to identify the molecular differences between zebrafish and mammalian NSPCs to utilize them for regenerative therapies. Multifaceted inflammatory conditions in neurodegenerative diseases affect

microglia, neurons, and NSPCs pleiotropically. Kizil et al. first developed a gene knockdown method based on cerebroventricular microinjection (CVMI) in vivo morpholino oligonucleotide in the adult zebrafish brain (Figure 2). CVMI injection in a skull incision is capable of uniformly targeting cells near the injection site, in this case the forebrain ventricular region containing neurogenic progenitor cells. The amyloid-β42 (Aβ42) induced neurotoxicity in adult zebrafish brain using CVMI of Aβ42 derivatives. One of the earliest findings in understanding the etiology of AD was the discovery of a 40-length peptide in AD brains now called Aβ, which constitute the primary component of AD-related amyloid deposits. Aβ is produced from the amyloid precursor protein (APP) with the continuous breakdown of β- and γ-secretases. Importantly, the creation of Aβ through APP's proteolytic processing is heterogeneous, leading to variable Aβ lengths, especially at the peptide's carboxy terminus. 40 and 42 long residues are the two main forms of Aβ produced under normal APP processing conditions (Aβ40 and Aβ42, respectively). The shorter variety of Aβ40 is the majority of the Aβ produced in a normal individual. Approximately 5%–15% of the total Aβ pool is Aβ42, and it is possible to observe smaller amounts of other Aβs, both longer and shorter. Generally, the brain's Aβ pool has 5%–15% of Aβ42, which causes reminiscent phenotypes of amyloid pathophysiology: apoptosis, microglial activation, synaptic degeneration deficiencies, and learning. Aβ42 also results in NSPC proliferation and increased neurogenesis . This understanding can help to design regenerative therapy-based drug discovery for neurological disorders.

2.9 Zebrafish Cell Culture-Based Neurodegenerative Disease Models

The developing zebrafish embryo is an excellent source for culturing cells, including neural cells. The technique to culture primary motor neuron (MN) in zebrafish has been developed for studying neurological disorders. The motor neuronal zebrafish cell culture was initiated at 24 hpf when the axonal development and outgrowth of MN started, allowing the development of MN axons in vivo in the context of the normal endogenous signs of the model organism, while also providing availability for an in vitro system. The zebrafish's primary culture techniques offer another approach to examine the neuronal population. There have been reports of primary neuron culture protocols ranging from blastula stage to 19 hpf , but these cultures are derived from MN axon pathfinding and neuromuscular development prior to normal course. Primary MN axons in zebrafish are present at 18 hpf out of the spinal cord, while the appearance of secondary MN axonal path findings range from 26 to 34 hpf. The brain explant cultures and primary cell culture of muscle fibers [150–152] are possible to develop from the later development stages of zebrafish embryogenesis. The advantages of primary zebrafish cell culture provide a new foundation to develop potential therapies for neurological disorders. A new protocol outlines how the subcellular spreading and protein aggregation status of neurodegenerative disease-causing neurons from transgenic zebrafish embryos can be investigated (Figure 3). ALS and spinocerebellar ataxia type-3 (SCA3) can be studied from this protocol, as the disease-causing sarcoma-fused (FUS) and ataxin-3 proteins of the human variant gene can be expressed in the zebrafish cell culture. A combination of neuronal subtypes, including motor neurons, exhibited cultural differentiation as well as an outgrowth of neurites. The human mutant FUS mislocated from nuclei to cytosol, imitating observed in human ALS and the zebrafish FUS model. In contrast, zebrafish-grown neurons expressing human ataxin-3 with disease-associated improved polyQ repeats did not build up in nuclei as frequently reported in SCA3. Another simple and efficient protocol was recently proposed to obtain the primary cells of embryonic zebrafish. By exploiting the cell-type rich resource specific fluorescent zebrafish reporter lines, different types of differentiated cells were cultivated and monitored, proving that they continued their original morphology in culture for many days and demonstrated that before cultivation, particular types of cells could be enriched with flow cytometry. This group also successfully tested several fluorescent vital colors to facilitate subcellular imaging. This technique delivers a new tool to enhance our understanding of neurodegenerative disorders pathogenesis and help the development of mechanism-based drugs for neurological disorders.
