Zebrafish as a Pharmacological Model: A Comprehensive Review

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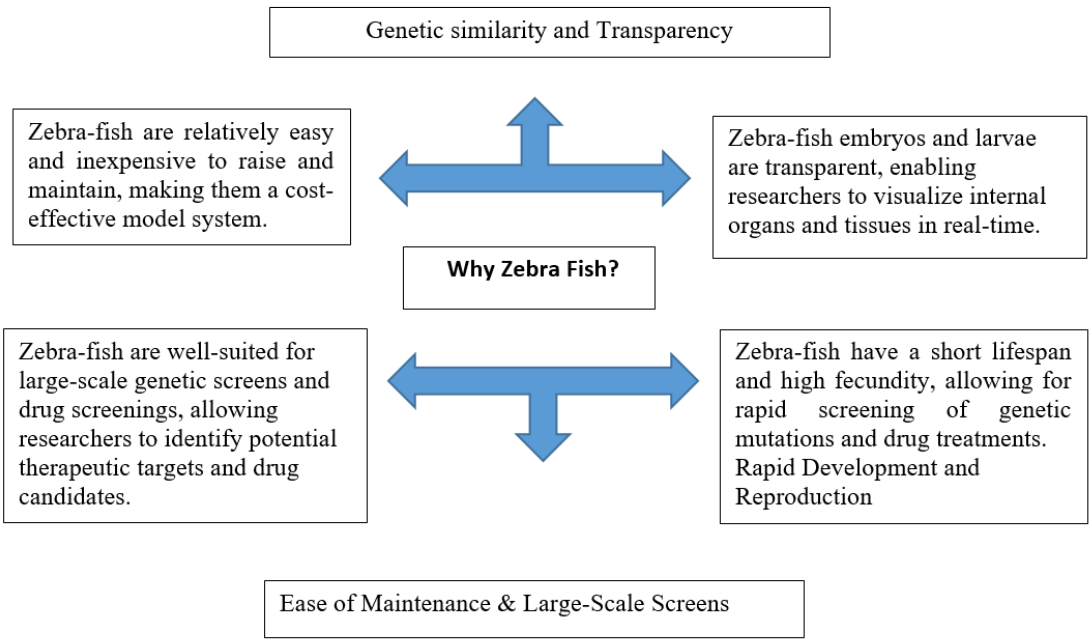
Abstract

Zebra fish (Danio rerio) has emerged as a versatile and valuable model organism in pharmacology, toxicology, and drug development. Due to its genetic similarities to humans, rapid development, and the ability to perform high-throughput screenings, zebrafish has become an increasingly popular tool for understanding drug mechanisms, studying disease models, and evaluating therapeutic efficacy. This review highlights the key features of zebrafish that make them an attractive pharmacological model, their use in drug discovery, their advantages over traditional models, and the challenges faced in their application.

Keywords: Zebra fish, Pharmacology, Genetic studies, Danio rerio, Toxicology Studies.

Graphical abstract



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Introduction

Model organisms have long been powerful tools for exploring human disease and biology. Owing to a remarkable degree of evolutionary conservation of fundamental cellular and molecular processes across species, both invertebrates and vertebrates have significantly contributed to biomedical research. Invertebrate models such as *Caenorhabditis elegans* and *Drosophila melanogaster* have been instrumental, particularly through the application of forward genetics. However, their lack of vertebrate-specific structures and organ systems—such as a skeleton, liver, kidneys, multi-chambered heart, multi-lineage hematopoietic system, and notochord—limits their utility in studying a wide range of human pathologies. Consequently, vertebrate models have become essential for investigating complex diseases and developing therapeutic interventions.

In biomedical research, the zebrafish (Danio rerio) has become a quite important and flexible creature among vertebrate models. Originally developed in the field of developmental biology, the zebrafish model has since grown into several fields, including pharmacology, toxicology, genetics, cancer, and neurology. Its special mix of advantages high fecundity, clear and open embryonic development, quick generation time, and simplicity of genetic modification helps to explain this change. Moreover, a significant model for investigating gene function and disease pathways is zebrafish as around 70% of human illness-associated genes have functional orthologs.

Zebrafish larvae and embryos offer a reasonably affordable, high-throughput, morally benign substitute for conventional mammalian models in pharmaceutical research. In a whole-organism environment, they provide insightful analysis of pharmacological effectiveness, toxicity, and pharmacodynamics, therefore enabling in vivo screening of therapeutic candidates. Their tiny scale and aquatic character help to administer compounds and monitor physiological changes in real-time, thereby improving the speed and efficiency of early-phase medication development [Avdesh, A., *et al* 2012].

All things considered, the zebrafish is a strong model system that closes the gap between in vitro research and higher-order mammalian models and provides a whole platform for knowledge of human disease and evaluation of new treatment approaches [Adhish, M., and Manjubala, I. 2023].

History

Beginning in the 1970s, George Streisinger, a pioneering geneticist at the University of Oregon who is also regarded as the founding father of zebrafish research by colleagues, helped the zebrafish to become a model organism. Streisinger realised that for direct real-time observation of developmental processes, zebrafish embryos, with their transparency and fast development, were perfect.

Scientific classification

Kingdom: Animalia

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Phylum: Chordata

Class: Actinopterygii (ray-finned fishes)

Order: Cypriniformes

Family: Cyprinidae (Minnows or carps)

Genus: Danio

Species: Danio rerio

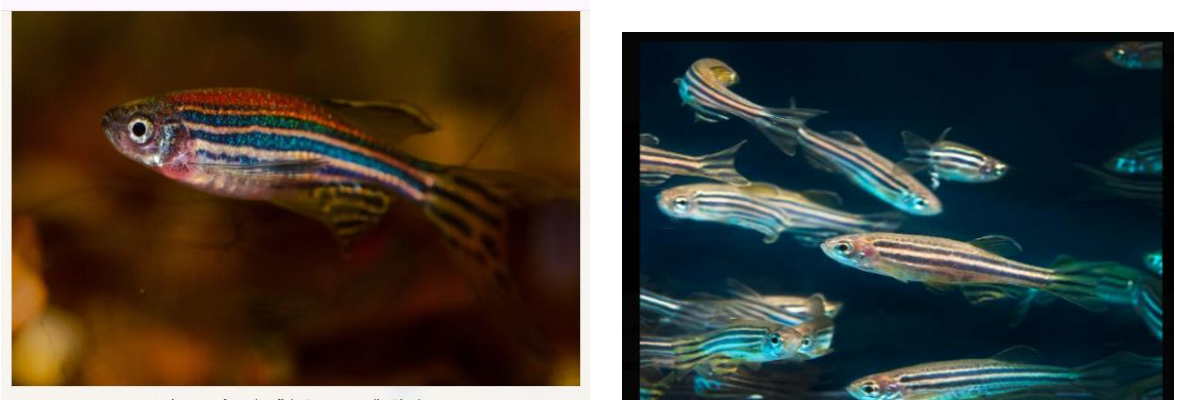

Figure 1: Zebra fish

- From the 1960s, the zebra fish was used as a model organism in clinical and pharmacological studies.
- The zebrafish is native to the tropical freshwater system of Southeast Asia.
- The zebra fish is a small vertebrate that is about 2.5 cm to 4 cm long.
- The zebra fish is transparent at its larval stage. When it matures into an adult, it develops the stripes which run along the length of the body, and it is blue.
- The zebra fish males have a more slender and torpedo-shaped body, and often exhibit a pink or yellow tinge.
- The zebra fish Females tend to be fatter due to they carry the eggs and are less pink than the males.
- The zebra fish have significantly contributed to unlocking several biological processes behind muscular dystrophy, & their genetic and physiological similarities to humans make them a valuable model for understanding the mechanisms of development & diseases such as cancer.
- In the year 2013, the full figured genomic sequence of zebra fish was published.
- The length of the zebra-fish genome is about 1,505,581,945 base pairs, and it contains 26,248 protein-coding genes.

Development of Genetic Tools

Streisinger cloned the zebrafish in 1981, therefore pioneering the cloning of a vertebrate. Using zebrafish, 2,3 Streisinger and associates also effectively performed the first mutagenesis to produce

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mutant strains fit for research on gene function. 4 Zebrafish became a model organism in great part due to the evolution of mutagenesis techniques and in vitro fertilization technologies during this era. These early discoveries made it possible for scientists to do methodical genomic searches and pinpoint genes crucial for development and illness [Cong, X., and Zon, L. I. 2010].

Expansion and Community Growth

Zebrafish research grew quickly throughout the 1990s. Large-scale mutagenesis screens and the creation of a zebrafish mutant library let scientists methodically investigate the genetic basis of many biological processes, therefore revealing many genes linked in embryonic development and illness. 06 Zebrafish study also spread during this time into disciplines like neuroscience, toxicology, and regenerative medicine. [Gaur, A., Mishra, R., Jain, S., and Jain, V. 2024].

Timeline: Zebrafish Adoption in Pharmaceutical Research

Year	**Milestone**
1990s	Large-scale forward genetic screening began in zebrafish
2000	Zebrafish genome sequencing project launched
2002	First drug screening platforms using zebrafish embryos
2010	CRISPR-Cas9 technology introduced for zebrafish gene editing
2015	Zebrafish models for neurological and cardiovascular diseases validated
2020+	Zebrafish are used in personalized medicine, oncology screening, and regenerative studies

Flowchart: Drug Discovery to Clinical Translation Using Zebrafish

1. Target Identification

Genetic studies: Disease pathways

2. Drug Discovery:

High-throughput screening in vitro & silico

3. Zebrafish Testing:

Phenotypic screening

Toxicity assays

Disease modeling

Behavior & organ function analysis

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4. Hit Validation & Optimization

Refine lead compounds based on zebrafish data

5. Mammalian Preclinical Testing

Mouse or rat studies

6. Clinical Trials (Human)

Phase I–III

The Zebra-Fish (Danio rario):

Reproduction in Zebra Fish:

Zebra fish undergo both fertilization and development externally, without parental care. Reproduction occurs in small groups, where the female releases her eggs into the substrate. Although sexual maturity is reached between ten and twelve weeks, starting reproduction at six months is recommended for optimal results and better-quality embryos. This species is notably small; mature zebra fish measure around 4-5 cm and display a cylindrical body with alternating light and dark horizontal stripes. They also show clear sexual dimorphism: males are rounder with a golden hue on their underside, while females are slimmer and more silver. This difference is particularly evident leading up to spawning. Females are asynchronous spawners, capable of spawning multiple times daily or every two to three days. Each spawning can yield up to 100 eggs, and a single female may produce as many as 200 eggs. Fry develop quickly and usually attain sexual maturity within two to three months.

Zebrafish as a Pharmacological Model

Zebrafish (*Danio rerio*) is a small tropical freshwater fish that belongs to the minnow family (Cyprinidae) of the order Cypriniformes. This model organism has emerged as a powerful tool for disease modeling and drug discovery due to its unique biological and genetic characteristics. Widely used in research for various diseases, including neurological disorders, cardiovascular diseases, liver disorders, and cancers, zebrafish have become a cornerstone in the field of biomedical science.

The zebrafish is a perfect model organism as it presents several benefits. First, zebrafish contain between 70% and 82% of human genes, and over 82% of genes linked to human disorders have orthologs in zebrafish. Second, their translucent, externally developing embryos let scientists see developmental events in real-time. Some genetically altered strains, including Casper, maintain transparency; they stay see-through even in maturity. Furthermore, fast maturing and very fecund, zebrafish attain sexual maturity in around three months and may generate 200–300 eggs per week.

Zebrafish need little lab space, are reasonably priced to keep, and are easy to handle. Their tiny nature qualifies them for high-throughput genetic testing and pharmacological screening. Behavioral evaluations, physiological parameter measurements like heart rate and blood flow, and real-time illness progression monitoring allow researchers to for forward and reverse genetics, developmental biology,

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toxicology, and behavioral neuroscience, Zebrafish are perfect because of their several benefits.

Because zebrafish are permeable to tiny compounds, one of their main advantages for pharmacological study is that Easy immersion in water allows drugs to be given, therefore enabling non-invasive testing. Oral dosing, microinjection, and intraperitoneal injection are among the several medication delivery techniques that zebrafish also accept. Crucially, their metabolic and pharmacokinetic characteristics closely match those of mammals, therefore supporting their use in preclinical research.

Many human disorders have been modeled using zebrafish. Their use spans neurological illnesses like Parkinson's and Alzheimer's, where they assist in the identification of neurotoxic and neuroprotective molecules. Zebrafish models in cardiovascular research reproduce features of congenital heart abnormalities and myocardial infarction. Transparency zebrafish lines like Casper let researchers in cancer studies see tumor development and metastases in real time. Models for liver, renal, and metabolic illnesses as well as for zebrafish, also abound.

Precise genome editing in zebrafish is made possible by genetic modification methods like TALENs, TILLING, and CRISpen-Cas9. These instruments have made it possible to build disease-specific models that quite faithfully reflect human pathophysiology. Because they have the unusual capacity to rebuild heart tissue, fins, spinal cord, and retina, zebrafish also greatly advance regenerative medicine. Investigating this regeneration potential helps one to find molecular and genetic processes applicable in human treatment.

Finally, the zebrafish has shown to be a rather flexible and useful model for investigating human illnesses and evaluating novel treatment approaches. Modern scientific research and customized medicine benefit much from its adaptability with high-throughput screening techniques, genetic resemblance to humans, and regenerative capacity.

Table 1: Anatomical Comparison: Zebrafish vs. Human Organs

Organ System	Human	Zebrafish	Comments
Brain	Complex, multi-lobed	Simplified, but structurally similar	Conserved neurotransmitter systems; used in neurodevelopmental studies
Heart	4-chambered (2 atria, 2 ventricles)	2-chambered (1 atrium, 1 ventricle)	Similar heart rate and physiology; good model for cardiotoxicity
Liver	Multi-lobed, complex	Single-lobed	Functional hepatocytes and bile ducts; used for liver toxicity screening
Kidney	Multi-nephron (metanephros)	Pronephros (larva), mesonephros (adult)	Conserved filtration and osmoregulation pathways

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Pancreas	Exocrine and endocrine lobes	Distinct exocrine and endocrine tissues	Glucose metabolism and insulin regulation conserved
Gut	Long, complex intestines	Short, simple gut	Similar absorption and digestion; useful in nutrient absorption studies
Eye	Complex retina	Cone-rich retina	Similar photoreceptor distribution; model for retinal and visual diseases
Skin	Stratified epidermis	Non-keratinized epithelium	Transparent; widely used in wound healing, regeneration, and toxicology

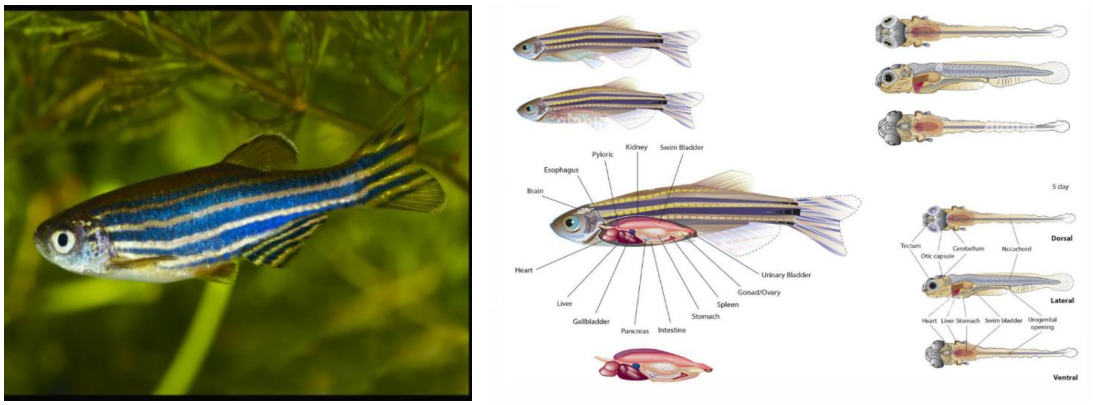

Figure 2 : Body parts and stages of Zebra fish

Applications of Zebra-fish in Pharmacology

1. Drug Screening and Discovery

Discovery of novel medicinal medicines has made great use of zebrafish. Their usage in HTS has helped numerous molecules with possible medicinal uses to be identified. Among these molecules are tiny ones meant for target cancer, pain, and inflammatory illnesses. Screening for molecules that can penetrate biological barriers—such as the blood-brain barrier, a major obstacle in medication development—zebrafish are very helpful. [Varshney, G. K., Sood, R., and Burgess, S. M. 2015].

2. Toxicology Studies

Another field for which zebrafish shine as a pharmacological model is toxicology. Their tiny scale and clear character make them perfect for assessing the toxicity of environmental contaminants, chemicals, and medications. Mammalian models have become less important as zebrafish larvae become a paradigm for acute toxicity testing. Effective for assessing medication safety, studies have revealed that zebrafish larvae display dose-dependent reactions to a variety of harmful chemicals.

3. Cardiovascular and Neuropharmacology

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Research on cardiovascular disorders and neuropharmacology is making greater use of zebrafish. Rapidly beating and easily watched in real-time, the zebrafish heart offers a great model for evaluating cardiotoxicity and cardiovascular medications. Neuropharmacological studies on the impact of medications on brain function—including antidepressants, antipsychotics, and neuroprotective agents—have also made use of zebrafish [Chakraborty, S., *et al*. 2022].

Examples of Human Diseases Studied in Zebrafish:

Cancer: Zebrafish models have been used to study various types of cancer, including Leukemia, melanoma, and rhabdomyosarcoma [Tiwari, R., Rathore, H., Mishra, R., and Jain, V. 2023].

Hematological Disorders: Zebrafish are used to study hematological disorders like Wiskott-Aldrich syndrome (WAS) and X-linked severe congenital neutropenia (XNS) [Zizioli, D., Mione, M., Varinelli, M., *et al*. 2019].

Neurological Disorders: Zebrafish models are used to study neurological disorders like Alzheimer's disease, epilepsy, and Amyotrophic Lateral Sclerosis (ALS) [Baraban, S. C., *et. al*, 2020].

Muscular dystrophy: Zebrafish models have been used to study Duchenne muscular dystrophy (DMD) and other muscular disorders [Arjmand, B. *et. al*, 2020].

Cardiovascular Diseases: Zebrafish are used to study cardiovascular diseases and metabolic disorders.

Viral Diseases: Zebrafish are used to study human viral diseases.

Dermatologic Diseases: Zebrafish have been increasingly utilized to model non-oncological skin diseases such as psoriasis, vitiligo, epidermal bullous, and chronic wounding.

Other Diseases: Zebrafish models are also used to study other human diseases, including pseudoxanthoma elasticum (PXE) and mitochondrial membrane protein-associated neurodegeneration (MPAN) [Boehm, U. *et. al*, 2015].

Advantages of Using Zebrafish Models:

Discover New Therapeutic Targets: Zebrafish models are valuable for identifying novel therapeutic targets and potential drug candidates for human diseases.

Speed up Drug Discovery: Zebrafish models can significantly speed up the drug discovery and development processes.

Investigate Disease Mechanisms: Researchers can utilize zebrafish models to gain insights into the mechanisms underlying human diseases. [White, R. M. *et. al*, 2021] .

Tailored Medicine: Zebrafish models facilitate the development of personalized medicine strategies for treating human conditions.

High-Throughput Screening: Zebrafish are ideal for conducting high-throughput screenings of small molecules and drug treatments.

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Ethical Aspects: Zebrafish are viewed as a more ethical choice than higher vertebrates for initial drug testing due to their small size and lower costs. Their use also decreases the reliance on mammalian models.

Genetic Alteration: Zebrafish are easily genetically modified, allowing for the investigation of gene functions related to disease and drug responses.

Economic Viability: Zebrafish are more cost-effective to maintain than mammals, making them a budget-friendly option for extensive screenings.

Live Monitoring: Their transparent bodies enable real-time observation of physiological changes, making them an excellent tool for studying drug effects. [Kulkarni, P., Chaudhari, G. H., Sripuram, V., *et al.* 2014].

Advantages of using the zebrafish

- The zebrafish is small yet physiologically resilient, making it ideal for experimental studies.
- They are less expensive to maintain than mice.
- Mating activity in zebrafish is typically initiated by the onset of daylight, which serves as an environmental cue.
- They reproduce hundreds of offspring at weekly intervals, which provides the scientists with an abundant supply of embryos to study.
- Zebrafish develop incredibly at a faster rate than a human embryo; a month to develop, they achieve in just one day.
- Zebrafish embryos are naturally transparent, allowing researchers to observe the development of internal organs and structures in real time. Every blood vessel in a living zebrafish embryo can be observed using a low-power microscope.
- The zebrafish lays its eggs outside the mother's body, making it an ideal model organism for studying early development.
- Zebrafish possess a remarkably similar genetic structure to humans; they share about 70% of our genes, making them a significant model for researching human biology and disease.
- Approximately 84% of the genes linked to human diseases are also present in zebrafish, enhancing their value as a model for medical research.
- As a vertebrates, zebrafish have the same major organs and tissues as humans. Their muscle, blood, kidneys, and eyes display many similarities to human systems.
- Zebrafish exhibit a remarkable ability; not only can they survive heart injuries, but they also regenerate the damaged portion. For instance, if a part of their heart is removed, it completely regenerates within a few weeks. This natural capability has piqued the interest of scientists, who are

 https://doi.org/10.5281/zenodo.15575852

now investigating the mechanisms behind it. The goal is to understand the specific factors involved in this healing process, aiming to apply similar methods to assist individuals suffering from heart attacks or heart failure.

- The complete sequencing of the zebrafish genome has been achieved with outstanding accuracy, providing researchers with a robust platform to explore gene function. As a result, scientists have introduced mutations in over 14,000 genes, allowing for investigation into how these genes affect development, physiology, and disease. [Bates, J. M., *et al*.,2007].

Limitations of using the zebrafish

Despite the many advantages, there are limitations to using zebrafish as a pharmacological model:

Lack of Human Complexity: While zebrafish share a significant amount of genetic homology with humans, they do not perfectly mimic the complexity of human disease. Therefore, findings in zebrafish models need to be validated in higher models.

Drug Metabolism Differences: Zebrafish do not have the same metabolic processes as humans, which can sometimes limit the predictive power of drug studies, particularly for compounds requiring human-specific metabolism.

Limited Models for Chronic Disease: Due to their rapid development, chronic disease models in zebrafish are less established than in mammals.

Zebra-fish frequently possess multiple copies of certain genes, known as paralogs, which can sometimes result in one or more of these copies being non-functional or having reduced activity. [Patton, E. E., and Tobin, D. M. 2019].

Low degrees of inbreeding in laboratory zebra-fish populations might affect their behavior, which can confuse the interpretation of data when investigating the consequences of certain genetic changes. Zebrafish flourish at a preferred temperature of about 28°C, which can provide challenges for studies needing circumstances nearer mammalian body temperatures.

Recent Advances and Applications in Zebrafish-Based Drug Discovery

Baraban et al., 2020: Showed that zebrafish models helped identify potential anticonvulsants using a *Dravet syndrome* model.

White et al., 2021: Used zebrafish to model melanoma and screened for small molecules targeting melanoma cells.

Chakraborty et al., 2022: Used CRISPR-generated zebrafish models to study *cardiomyopathy* and validated a potential treatment.

Emerging Genetic Technologies Empowering Zebrafish as a Next-Gen Disease Model:

Over the past decade, zebrafish (Danio rerio) have transitioned from simple developmental models to powerful systems for genetic and disease research. This transformation has been largely driven by the

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advent of advanced genetic manipulation tools, which allow for precise modeling of human diseases, targeted gene editing, and functional genomics studies [Xu, C., and Zon, L. I. 2010].

1. CRISPR-Cas9 Genome Editing

The CRISPR-Cas9 system has revolutionized zebrafish research by enabling:

- Precise gene knockouts to study gene function.
- Point mutations to mimic human genetic disorders.
- Conditional or tissue-specific editing for targeted investigations.

Example: Mutations in the pten gene induced via CRISPR in zebrafish have provided valuable models for studying tumorigenesis and metabolic disorders.

CRISPR's simplicity, high efficiency, and cost-effectiveness make it the most widely adopted gene editing method in zebrafish today [Choi, T. Y., Choi, T. I., Lee, Y. R., *et al.* 2021]

2. TALENs and ZFNs (Transcription Activator-Like Effector Nucleases .& Zinc Finger Nucleases)

CRISPR, TALENs and ZFNs were used for targeted gene disruption previously. Although they require complex protein engineering, they are still useful for:

- Generating large deletions or insertions.
- Avoiding potential off-target effects sometimes associated with CRISPR.

These tools remain valuable in cases where high specificity is crucial.

3. **Morpholino Oligonucleotides**

- Morpholinos are synthetic molecules used to transiently block mRNA translation or splicing.
- Ideal for early embryonic studies.
- Frequently used in gene knockdown experiments.

However, morpholinos are limited by:

- Short-lived effects, as they degrade quickly.
- Potential off-target toxicity, making proper controls essential.

Despite their limitations, morpholinos remain useful for validating CRISPR results during early development.

4. **Transgenesis and Reporter Lines**

Zebrafish are highly amenable to stable transgenesis. Researchers commonly create:

- Tissue-specific fluorescent reporter lines (e.g., GFP-tagged liver, heart, or neurons).
- Drug-inducible expression systems (e.g., Tet-On/Tet-Off, Gal4/UAS).
- Cre-loxP-based conditional expression systems.

These tools allow:

- Real-time visualization of gene expression.

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- Tracing of cell lineages.
- Targeted expression of human disease genes.

5. Gene Trap and Enhancer Trap Techniques

These tools help identify:

- Unknown gene functions.
- Regulatory elements that control gene expression.
- Through random insertion of reporters, researchers can observe dynamic gene activity patterns during development or disease progression.

6. **Optogenetics and Chemogenetics**

Emerging tools like optogenetics and chemogenetics in zebrafish are enabling:

- Precise control of neuronal activity using light (optogenetics).
- Cell-specific modulation using designer receptors activated by drugs (chemogenetics).

These technologies allow real-time functional analysis of circuits involved in behavior, cognition, and disease.

7. Epigenetic Editing Tools

New CRISPR-based systems (e.g., CRISPR-dCas9) fused to epigenetic modifiers can alter gene expression without changing DNA sequences. These tools open avenues to study:

- DNA methylation.
- Histone modifications.
- Non-coding RNA regulation.

Such approaches are invaluable in exploring complex gene-environment interactions.

Conclusion

Zebrafish have become a valuable tool in pharmacological research due to their fast development, genetic similarity to humans, and ability to serve as a platform for high-throughput drug testing. While they are not a perfect replacement for mammalian models, they offer an ethical, cost-effective, and efficient way to study drug efficacy, toxicity, and disease. With further advancements in zebrafish research, they are poised to play an even more critical role in the future of drug development and medical research.

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