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Zebrafish: A Genetic Wonder Revealing Regeneration and Drug Discovery Secrets

Sayed Mohammed Firdous[1], *Sourav Pal*[2], *Sushmita Mohato*[1]

[1]Department of Pharmacology, Calcutta Institute of Pharmaceutical Technology & AHS, Uluberia, Howrah: 711316, West Bengal, India. [2]P.G. Institute of Medical Sciences, Dhurabila, Dhamkuria, Chandrakona Town, Paschim Medinipur: 721201, West Bengal, India

[*]Correspondance author**:** firdous.oncology@gmail.com

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Abstract

The purpose of this review is to draw attention to the many ways in which zebrafish (*Danio rerio*) have been used in biomedical research, particularly in the areas of drug development, regeneration processes, and pathology. Many benefits are associated with zebrafish, such as their tiny size, quick growth, transparency throughout early development, and genetic resemblance to humans. In the field of drug development, they are highly useful for assessing the effectiveness, safety, and adverse effects of drugs, especially in the context of cancer, neurological illnesses, and cardiovascular disease. Brain diseases like addiction and anxiety can be better understood with the help of zebrafish, which are also useful in neurobehavioral research. A wide range of diseases, including those affecting the blood and bones, can be better understood and treated with the use of zebrafish models. These models are also excellent for discovering new drug targets and evaluating the efficacy of possible treatments. Zebrafish can also repair their own fins, hearts, spinal cords, and even their eyes if they become injured. by controlling key signalling cascades like Wnt, Hippo, and FGF, new therapeutic targets have been identified. This renders them priceless for investigations into human tissue injury and organ failure, as well as investigations into mechanisms of regeneration. Researchers still need to think about the limits of zebrafish models, like genetic duplications, physiological and anatomical variances, and the limitations of antibody-based techniques, even though they are useful. Ensuring the applicability of research outputs to human health and illness requires a thorough understanding of these limitations.

Keywords: Zebrafish, Hippo signaling pathway, Wnt pathway, Mef2 pathway, FGF pathway

INTRODUCTION

In accordance with the multitude of advantages they serve, zebrafish (*Danio rerio*) have become an extensively utilized model organism in the process of developing new drugs as well as performing research on neurobehavioral processes [1]. These advantages include small dimensions, swift growth, and high fecundity, as well as their transparency during the early stages of development, which allows for easy sight of interior organs and structures [2]. Additionally, their genetic similarity to humans makes them a useful model for studying human disease [3]. Zebrafish are particularly useful in drug development because they share many genetic cascades with humans, and many drugs that work in zebrafish have been found to work in humans as well [3,4]. Zebrafish models have been used to study drug effects on various conditions, including cancer [5], neurological disorders [6], and cardiovascular disease [7]. The use of zebrafish in drug development allows for the identification of potential drug targets and the testing of drug efficacy, toxicity, and side effects [8]. In neurobehavioral analysis, research on the behavioral effects of drugs and other substances is often conducted using zebrafish as the subject animal. Zebrafish exhibit complex behaviors such as social interactions, shoaling, and predator avoidance, making them a useful model for studying the effects of drugs on behavior [9]. Additionally, zebrafish have a similar nervous system to humans and exhibit behaviors similar to those seen in humans, making them a useful model for studying human neurological disorders such as anxiety, depression, and addiction [10]. They are widely used in different studies like neurobehavioral assessment, pharmacology, and other diseases related to humans [11]. Their application in the evaluation of the

neurobehavioral effects of pharmacological substances has seen a significant uptick as of late. The term "neurobehavioral" refers to the specific kind of disruptive behaviors that are brought on by conditions affecting the brain [12].

Neurobehavioral disorders refer to a wide range of conditions that affect the brain and nervous system, leading to alterations in behavior, cognition, and emotions [13]. These disorders can be caused by various factors, including genetic, environmental, and lifestyle factors. Examples of neurobehavioral challenges include amyloid-β plaque, hyper-phosphorylated tau protein, α-synuclein aggregation, ADHD, and anxiety disorders [14]. The purpose of this review is to draw prominence to the numerous circumstances in which zebrafish have been used as an animal model, particularly in the fields of drug discovery, regeneration mechanisms, and the study of biological processes.

As a model organism, zebrafish provide certain benefits to biological studies. Their manageable length of about 3–4 cm makes them ideal for use in laboratories where space is at a premium. Their embryos are also see-through, so researchers can monitor their progress in real time without harming the embryos [15]. A zebrafish embryo forms its main organs and structures just a few days after fertilization, making it an ideal model for studying embryogenesis in a short amount of time [16]. Because they share so many genes with humans, zebrafish are a great model organism to study genetic abnormalities and human illness [3,17]. They show great potential for regenerative medicine due to their extraordinary regeneration powers, especially in tissues of the heart and spinal cord [18]. Zebrafish are a cost-effective model organism for scientific inquiries because they are easy to breed under laboratory conditions [19]. Because they can lay hundreds of eggs in only one mating, their great fecundity guarantees an abundance of embryos for research [20]. To speed up the process of drug discovery, zebrafish embryos are ideal for high-throughput screening because of their tiny size and quick development.

The lifespan of zebrafish is 3.5 years but some zebrafish live up to 5.5 years [21]. Zebrafish have the same ability to exercise as mammals with age. It has been observed in the organism's performance and training ability as it ages (Figure 1).

Some of the main stages of the lifecycle of a zebrafish:

The life cycle of zebrafish can be divided into several distinct stages, including:

Embryonic stage: The embryonic stage begins with fertilization, where the egg is fertilized by sperm to form a single cell. The cell will divide very quickly during the course of the following few hours, finally producing a sphere of cells that will develop into a blastula. By 24 hours after fertilization, the embryo has formed a distinct body plan, with a head and tail, and is beginning to develop major organs [22].

Larval stage: The larval stage begins around 48 hours after fertilization when the embryo hatches from its egg casing. At this stage, the larva is about 2-3 mm in length and has a transparent body. Over the next few days, the larva grows rapidly and begins to feed on small organisms, such as rotifers and paramecia [23].

Juvenile stage: The juvenile stage begins around 2-3 weeks after fertilization when the larva has grown to about 6-8 mm in length and has developed all of its major organs. At this stage, the zebrafish can be sexed by examining the gonads under a microscope. The juvenile fish continue to grow and mature over the next few months [24].

Adult stage: The adult stage is reached around 3-4 months after fertilization when the zebrafish has reached its full size, which is typically around 3-4 cm in length. At this stage, the zebrafish is sexually mature and capable of reproducing. The lifespan of zebrafish is around 2-3 years, although this can vary depending on the environmental conditions and genetic background of the fish [25].

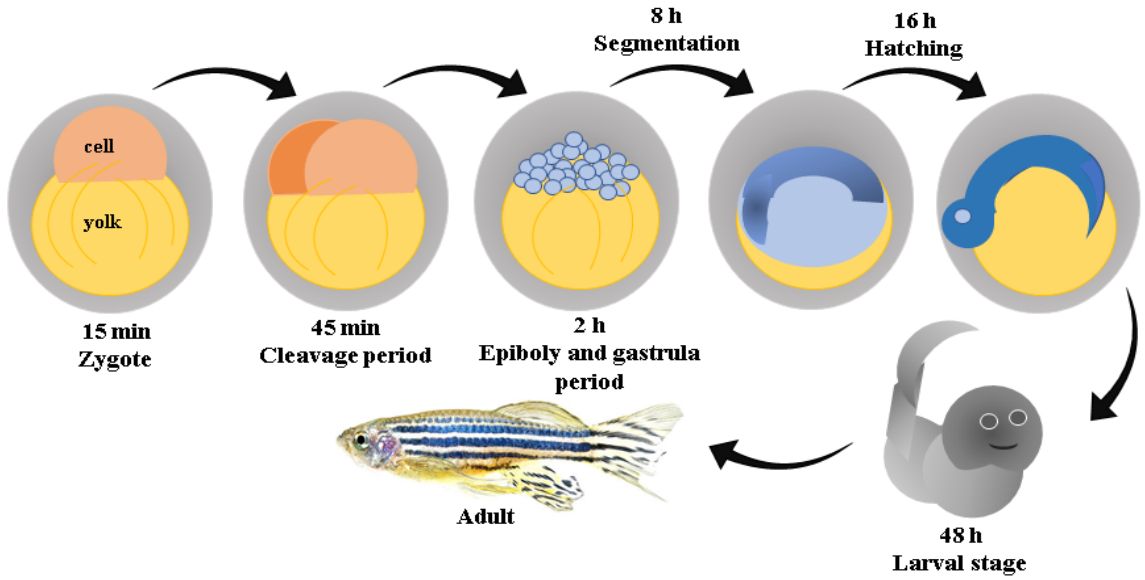

Figure (1): Life Cycle of Zebrafish.

The zebrafish embryo progresses through distinct developmental stages post-fertilization. Starting with the zygote, rapid cell division occurs for 15 minutes, followed by a 45-minute cleavage period forming a blastula. Epiboly and gastrula formation follow for 2 hours, then 8 hours of segmentation. After 16 hours, hatching occurs, leading to a 48-hour larval stage, culminating in adult zebrafish development with characteristic features.

Breeding of zebrafish

Breeding zebrafish is relatively straightforward and can be carried out in a laboratory setting. Careful consideration of environmental factors is required while setting up breeding tanks for zebrafish. To keep zebrafish in an environment similar to their native habitat, aquariums usually contain clean, oxygenated water kept at a temperature of 28 to 30 ℃. To avoid mating too soon, it's best to keep the men and females in separate aquariums [26]. It is possible to improve mating success by introducing one or two females for every male once they have acclimated. The behavior of mating must be carefully observed. The process of fertilization takes place in the water when male zebrafish pursue females and gently tap their bellies to encourage egg release. After mating has taken place, the partition is taken down so the tank can reproduce freely. Adhering to tank surfaces or carefully positioned breeding materials is a common method for collecting sticky zebrafish eggs, which takes skill. Gently collect the eggs with a fine mesh net and place them in an incubator. If the zebrafish eggs need to hatch, it's necessary to incubate them for at least 48 to 72 hours [27]. At this time, it is very important to keep the water at a consistent temperature and quality. Until they reach a size where they can eat commercial fish food, fry care includes feeding them appropriate foods like rotifers and newly hatched brine shrimp. To keep the zebrafish fry healthy and promote their development, water temperature, and quality must be carefully monitored.

Ideal housing conditions for zebrafish

It is dependent on several different aspects, such as the age and size of the fish, the goals of the research, and the resources that are readily accessible. Zebrafish in captivity require meticulous management of several critical variables to guarantee their health and growth. First and foremost, make sure the tank is big enough for the zebrafish to swim around and interact with each other like they would in the wild. It is generally recommended to have a tank size of 2-5 gallons per adult fish to meet their needs [28]. Because zebrafish are so delicate, it is critical to keep the water they live in clean at all times. To keep them in an environment similar to their native habitat, the water needs to be oxygenated, kept at a temperature of 28 to 30

°C, and a pH range of 7.0 to 7.5 [29]. For water to be of the highest quality, it must be filtered thoroughly to remove impurities and dangerous microorganisms. In most cases, a mix of mechanical, biological, and chemical filtering techniques is employed. To control their circadian cycles and behavior, zebrafish also need a regular light-dark cycle. They usually follow a 14-hour light and 10-hour dark schedule. To keep zebrafish from being stressed out or bored, it's important to provide them with an environment that encourages their natural behaviors by adding things like pebbles, plants, hiding spots, and food riddles. Optimal living conditions and the long-term health of zebrafish populations can be maintained by routine maintenance operations such as water changes, tank cleaning, and equipment upkeep.

Zebrafish provide 3Rs value to drug discovery toxicology

Zebrafish have become an increasingly popular model organism in drug discovery and toxicology due to their unique characteristics that provide 3Rs (reduction, refinement, and replacement) value [30].

Reduction: Zebrafish are tiny, easy to maintain, and have rapid reproduction, making them an ideal model organism for reducing the number of animals used in drug discovery and toxicology studies.

Refinement: Zebrafish have a highly developed cardiovascular system, which is similar to that of humans. This allows researchers to explore the effects of drugs on the heart and circulation in a more refined and precise way than using traditional animal models.

Replacement: Zebrafish can be used as a replacement for mammals in some studies. For example, zebrafish have been used to study the toxicity of chemicals and drugs on embryos, which would typically be done in mammals such as rats or rabbits.

The emergence of the zebrafish diseases model

Numerous human diseases, including malignancy, abnormal cell growth, cardiovascular disease, neurological disorders, and genetic disorders, have been studied using zebrafish [31]. For example, zebrafish have been used to identify potential drug targets for cancer and to test the efficacy of cancer drugs [32]. They have also been used to study heart disease and to test potential therapies for heart failure [33]. Zebrafish have also been used to study genetic disorders, such as cystic fibrosis and muscular dystrophy [34]. One example of a zebrafish disease model is the human genetic disorder cystic fibrosis (CF). Zebrafish with a mutation in the CFTR gene, which is associated with CF, have been developed [35]. These zebrafish display symptoms similar to those seen in humans with CF, such as a build-up of mucus in the airways. Researchers have used these zebrafish to study disease mechanisms and test potential therapies, such as gene therapy and drug treatments.

Ailments concerning osseous tissues

Understanding human bone diseases, such as osteoporosis and osteogenesis imperfecta (OI), is made much easier with the use of zebrafish models. Zebrafish provide a one-of-a-kind model for investigating skeletal diseases due to their haploid genome, which contains 25 chromosomes and 1.7 billion base pairs [36]. Using forward and reverse genetic approaches, researchers have generated mutant lines that mimic human skeletal diseases. Researchers may investigate how disease mutations affect gene expression, cell differentiation, and signaling cascades in vivo using zebrafish models because of their adaptability. The pathophysiology of osteoporosis can be better understood by manipulating gene expression associated with the mineralization of the extracellular matrix, signaling cascades, and the function of osteoblasts and osteoclasts [37]. Additionally, zebrafish models make it easier to study GIOP, a disorder characterized by low bone mineral density as a result of an imbalance in bone production, which is caused by glucocorticoid use [38]. When it comes to osteogenesis imperfecta, zebrafish models are really helpful. Fractures are more common in the *chihuahua* (chi) zebrafish mutant, which mimics human OI symptoms [39]. Zebrafish models are essential for understanding how changes in gene expression affect bone health and osteoporosis, according to the research. Research has identified several genes that play

critical roles in the function of osteoblasts and osteoclasts; their dysregulation leads to osteoporosis in zebrafish. These genes include entpd5a, acp5a, sost, mmp9, and mmp13 [40]. Bone mineral density decreases, microarchitecture changes and fragility rises as a result of these changes. In addition, the processes of glucocorticoid-induced osteoporosis can be better understood using zebrafish models. Osteoporotic alterations, such as increased activity of osteoclasts, resorption of matrix, and changes in gene expression profiles, are induced by prednisolone administration during scale regeneration [41]. This highlights the value of zebrafish models in evaluating therapeutic approaches for bone disorders, as alendronate treatment partially cures these abnormalities.

Ailments concerning hematopoiesis and related diseases

Zebrafish provide a great opportunity for exploring hematopoiesis and related diseases because of their human-like genetic makeup and their capacity to undergo similar processes in the development of the main blood cell types [42]. Zebrafish are incredibly useful for studying and treating diseases like leukemia because they are similar to transcription factors, signaling molecules, and functional proteins. Scientists have uncovered new information about blood ailments by using zebrafish models. As an example, zebrafish that carried the *spi1* mutant allele had symptoms similar to those of AML and myelodysplastic syndrome (MDS) in humans, including an excess of immature granulocytes and an accumulation of myeloblasts in the peripheral circulation [43]. This model is useful for testing therapies since its sensitivity to cytarabine and other chemotherapeutic drugs is similar to that of human AML patients [44]. Researchers at UCL are studying zebrafish to learn more about MDS and Diamond-Blackfan anemia (DBA), two types of blood diseases [45]. To better understand the genetic causes of DBA and MDS and to create new treatments, scientists are using zebrafish models that have had the ribosomal protein genes (Rps19, Rps26, Rpl11, and Rps14) knocked down [46]. Concerning ribosomal protein-mediated blood disorders such as DBA and 5q-MDS, their research aims to determine the function of aberrant translation [47]. They seek novel therapeutic candidates through the utilization of high-content screening on zebrafish embryos. As an additional step, they intend to conduct clinical trials to validate these findings in human cells.

Assessing oncogenic risk through toxicological studies

The validity of zebrafish models is supported by the fact that tumors developed in these animals resemble human tumors both histologically and molecularly. Scientists have developed zebrafish models to investigate cancer risk factors, such as Li-Fraumeni syndrome, familial adenomatous polyposis, RASopathies, and inherited syndromes of bone marrow failure [48]. Genome editing tools in zebrafish are very specific and flexible, which has greatly improved our ability to study cancer risk syndromes. Studies on the lung cancer microenvironment, medication toxicity testing, screening for antiangiogenic medicines, target identification, validation, cancer proliferation, and metastasis have all made use of zebrafish in the field of lung cancer research [49]. Zebrafish models have been utilized for in vivo tracking of NSCLC lesions and to enable personalized medication approaches [49,50]. Cancer development may be studied more easily using zebrafish since their eggs and larvae are transparent, allowing researchers to see the growth and dynamics of tumor cells in real-time [51]. Tumorigenesis relies heavily on mutated cancer driver genes, and zebrafish have been induced to develop cancer using techniques such as chemical mutagenesis and irradiation mutagenesis [52].

Researchers have also utilized zebrafish models to examine the tumor microenvironment (TME), a complex network of cells, proteins, and soluble substances that influence tumor characteristics [53]. The capacity to observe the interplay between cancer cells and different cells in the TME is a notable strength of the zebrafish model. For these reasons, zebrafish are a great model to study cancer in because they can shed light on tumor biology, the tumor microenvironment, and cancer susceptibility syndromes. According to Shen et al. (2023), kaempferol effectively suppresses the growth of intersegmental vessels (ISVs) in zebrafish

embryos at a concentration of 40 µM. Similarly, deoxypodophyllotoxin, derived from the roots of Anthriscus sylvestris, demonstrates significant inhibition of ISV growth in zebrafish embryos at a concentration of 50 nM. These findings highlight the potential anti-angiogenic properties of kaempferol and deoxypodophyllotoxin, suggesting their utility as candidates for further investigation in combating angiogenesis-related disorders, including cancer, utilizing zebrafish as a model organism [54].

Using zebrafish xenograft models, researchers have found that fucoidan and osthole are effective in ovarian cancer research. Fucoidan, a compound derived from *Fucus vesiculosus*, reduced tumor size and suppressed tumor growth in OV90 and ES2 cells [55]. Both cell lines showed a marked reduction in tumor development when exposed to osteohole, which was extracted from different plants. Similarly, zebrafish xenografts of ovarian cancer showed anti-cancer benefits when administered fucosterol, eupatilin, or cratoxylumxanthone C. Zebrafish models of non-small cell lung cancer showed that xipsxanthone H and cardenolide glucoevatromonoside reduced tumor cell migration and proliferation [56]. Research on breast cancer has shown that fucoidan, actein, betulinic acid, and jadomycin B all have anti-tumor properties. Oridonin and furanodiene, when used for liver cancer, prevented tumor growth in zebrafish xenografts [56]. Sarinosterol acetate also showed anti-cancer effects in zebrafish xenograft models of liver cancer. Melanoma cells in zebrafish xenografts were inhibited by theaflavin and shikonin in a dose-dependent manner [57]. Research on possible anti-cancer drugs and their modes of action can be aided by using zebrafish xenograft models, as demonstrated in these studies.

Neurodegenerative disease

Zebrafish have been used to model several neurodegenerative disorders, such as Tau protein hyper-phosphorylation, amyloid-β plaque, aggregation of α-synuclein, and CAG trinucleotide repeat in the HD gene are markers for the development of neurological artifacts (Figure 2) [58]. Here are some examples of how zebrafish have been used in the study of neurodegenerative diseases:

Alzheimer's disease (AD): The effects of beta-amyloid, which clumps into the brains of Alzheimer's sufferers, have been studied using zebrafish to investigate how it affects neuronal activity. Researchers have also employed zebrafish to investigate the function that inflammation plays in AD [59]. The fact that zebrafish share orthologs of important Alzheimer's genes (e.g., PSEN1, PSEN2, APP, and ApoE) makes them valuable models for studying disease processes [60]. A model for early-onset Alzheimer's disease can be found in mutations in orthologs in zebrafish, such as psen1, psen2, and app. As an example, the EOfAD alterations in humans are mirrored by the Q96_K97del psen1 mutation [61]. Zebrafish provide several benefits, such as a completely sequenced genome, quick development, and transparency, which can be used to screen drugs and genetics for potential treatments for AD.

Parkinson's disease: Research on the effects of alpha-synuclein, a protein that builds up in the brains of people with Parkinson's disease, on the functioning of neurons has been conducted using zebrafish. Researchers have also employed zebrafish to investigate the impact that oxidative assault plays on Parkinson's disease [62]. Similar to the pathology seen in Parkinson's disease, zebrafish exposed to MPTP show neuron loss in the ventral diencephalon. Similar to motor deficiencies seen in Parkinson's disease, this causes a diminution of the swimming speed [63].

Huntington's disease: Zebrafish have been used to study the effects of the mutant huntingtin protein, which causes Huntington's disease, on neuronal function. Zebrafish have also been used to study the effects of various drugs on the mutant huntingtin protein [64].

Amyotrophic lateral sclerosis: Zebrafish have been used to study the role of the protein TDP-43, which is implicated in ALS, on neuronal function. Zebrafish have also been used to study the effects of various drugs on TDP-43 [65].

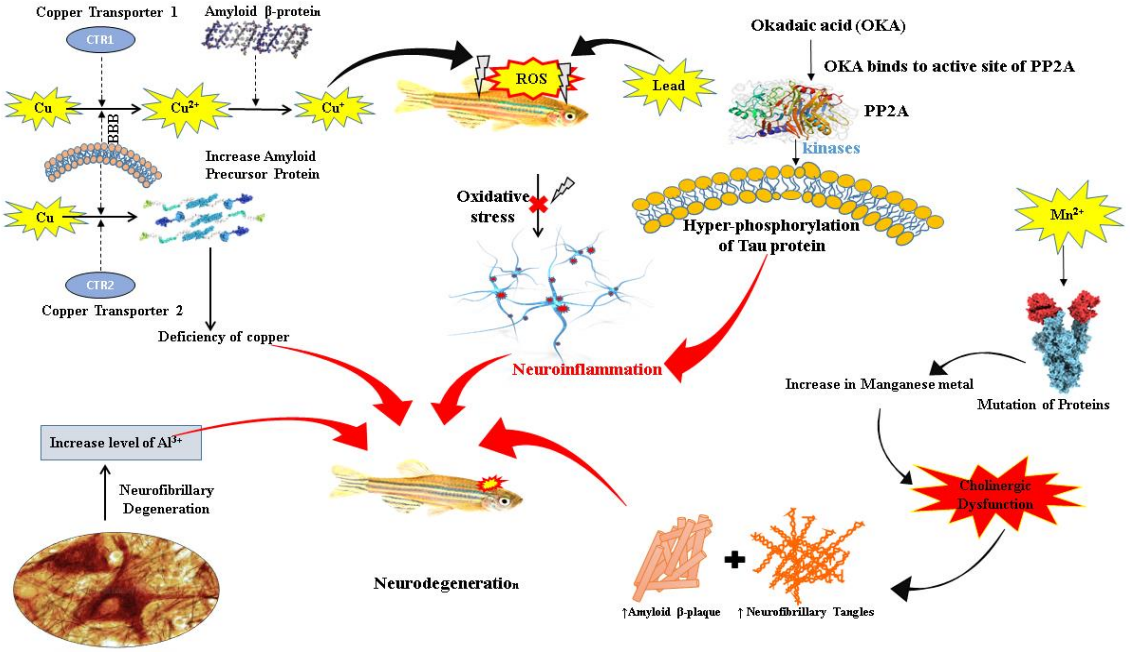

Figure (2): Zebrafish in Neurodegenerative Disease.

The pathophysiology of neurodegenerative diseases in zebrafish can be better understood by studying the roles played by oxidative assault, amyloid-beta, PP2A dysregulation induced by OKA, and Tau hyper-phosphorylation. An excess of aluminum and a deficiency of copper resulting from a lack of copper transporter 2 exacerbate neurofibrillary degeneration and inflammation. Neurodegeneration, characterized by neurofibrillary tangles and amyloid plaques, is caused by protein mutations.

Regeneration

Zebrafish have a remarkable ability to regenerate damaged tissues and organs, which makes them a valuable model organism for studying regeneration. Here are some examples of how zebrafish can regenerate different tissues and organs:

Fin regeneration: Zebrafish can completely regenerate their fins, including bones, blood vessels, and nerves, after amputation. This process involves the formation of a blastema, which is a group of undifferentiated cells that proliferate and differentiate into the various tissues of the fin [66].

Heart regeneration: Zebrafish can regenerate heart tissue after injury. This process involves the proliferation and differentiation of cardiomyocytes (heart muscle cells) from a pre-existing pool of cardiomyocytes [67].

Spinal cord regeneration: Zebrafish can regenerate spinal cord tissue after injury. This process involves the proliferation and differentiation of glial cells and the reconnection of severed axons [68].

Eye regeneration: Zebrafish can regenerate damaged or lost retinal neurons. This process involves the proliferation and differentiation of retinal progenitor cells [69].

The study of zebrafish regeneration has the potential to lead to new therapies for human diseases and injuries that involve tissue or organ damage. The molecular mechanisms underlying angiogenesis and regeneration in zebrafish are complex and involve a variety of cellular processes and signaling cascades. A phenomenon known as angiogenesis involves the creation of new blood vessels in zebrafish embryos [70]. This process is regulated by several signaling channels, some of which are the transforming growth factor beta (TGF-β) cascade, the Notch cascade, and the vascular endothelial growth factor (VEGF) cascade [71]. These cascades communicate with one another to encourage the proliferation, migration, and differentiation of endothelial cells, which eventually results in the development of new blood vessels [72]. The heart regeneration process of zebrafish

involves the activation of the Hippo signaling cascade, which promotes cardiomyocyte proliferation, and the Mef2 transcription factor, which is involved in cardiac muscle differentiation [73]. Zebrafish can regenerate their fins after amputation, and this process involves the formation of a blastema, which is a group of undifferentiated cells that differentiate into the various tissues of the fin. The formation of the blastema is regulated by various signaling cascades, including the Wnt, FGF, and BMP cascades, which promote the proliferation and differentiation of the blastemal cells [74]. Zebrafish can regenerate their spinal cord regeneration process which involves the activation of the Notch cascade, which is involved in glial cell proliferation and differentiation, and the Wnt and BMP cascades, which promote axonal regeneration and the formation of new synapses [75].

Heart regeneration

Although zebrafish and humans are separated by about 450 million years of evolution, they share many similarities in terms of heart development and function at the molecular level. Here are some of the key molecular mechanisms that are conserved between zebrafish and humans:

Many of the transcription factors that regulate heart development and function are conserved between zebrafish and humans. For example, the Nkx2.5 transcription factor is crucial for cardiac progenitor cell specification and differentiation in both zebrafish and humans [76]. There is a high degree of similarity between zebrafish and humans in terms of the signaling cascades that control the growth and function of the heart. For example, the BMP and FGF signaling cascades are both involved in regulating cardiomyocyte proliferation and differentiation in zebrafish and humans [77]. Cardiac ion channel: Many of the ionotropic receptors that are responsible for generating and regulating the electrical activity of the heart are conserved between zebrafish and humans. For example, the potassium channel KCNQ1 is involved in regulating cardiac repolarization in both zebrafish and humans [78]. The sarcomeres, which are the basic contractile units of muscle cells, are highly conserved between zebrafish and humans [79]. Both zebrafish and humans have similar sarcomere structures and regulatory proteins that are involved in regulating the contraction and relaxation of cardiac muscle cells [80].

The molecular mechanisms of heart regeneration

In zebrafish, it involves a complex interplay of cellular processes and signaling cascades. Here are some of the key molecular mechanisms involved:

Activation of the Hippo signaling cascade

In zebrafish, the Hippo cascade is activated after heart injury and promotes cardiomyocyte proliferation through the activation of the transcriptional co-activator Yap [81]. The molecular mechanism of the Hippo signaling cascade in the heart regeneration of zebrafish involves a cascade of events initiated by the activation of the upstream kinase MST1/2, which in turn activates the downstream kinase LATS1/2 [82]. LATS1/2 then phosphorylates and inactivates the transcriptional co-activators YAP and TAZ, preventing them from translocating to the nucleus and activating downstream target genes [83]. In response to injury, the Hippo signaling cascade is downregulated, allowing YAP and TAZ to translocate to the nucleus and activate downstream target genes that promote cardiomyocyte proliferation and heart regeneration (Figure 3) [84]. The downstream targets of YAP and TAZ include genes involved in cell cycle progression, such as cyclin D1 and E, as well as genes involved in cell survival, such as Bcl-2 and surviving [85].

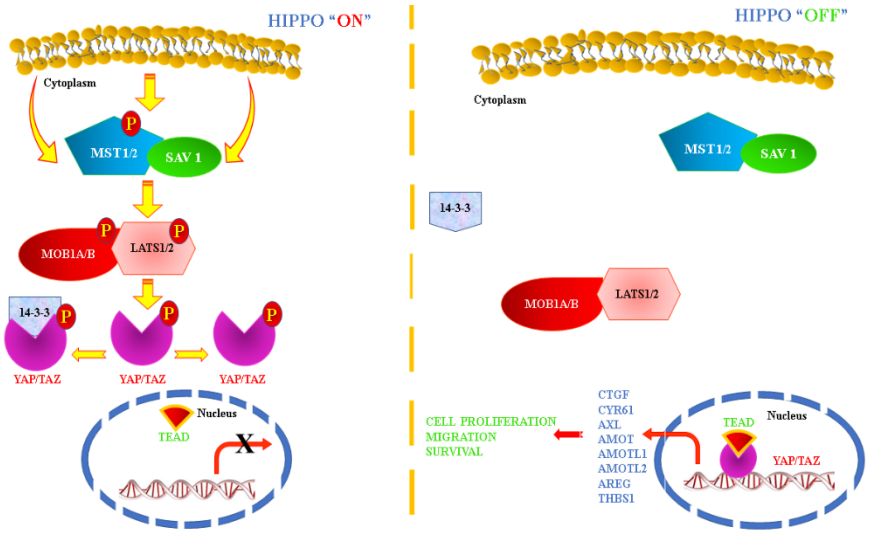

Figure (3). Schematic representation of the Hippo signaling cascade-YAP/TAZ.

When the Hippo signaling cascade is active/on (Left), multiple upstream signals regulate the phosphorylation of MST1/MST2, LATS1/LATS2 kinases, and phosphorylates YAP/TAZ proteins. Phosphorylation of YAP/TAZ recruits 14-3-3 proteins that stimulate cytoplasmic retention or proteolytic degradation. When the Hippo signaling cascade is inactive/off (Right), YAP/TAZ is not phosphorylated, localizes to the nucleus, forms a complex with transcription factor TEADs, and regulates genes required for endothelial cell proliferation, migration, and survival (Figure 3).

Activation of the Wnt signaling cascade

The Wnt signaling cascade is involved in various cellular processes, including cell proliferation and differentiation [86]. In zebrafish, the Wnt cascade is activated after heart injury and promotes the proliferation of cardiomyocytes and the formation of new heart tissue [87]. The molecular mechanism of the Wnt signaling cascade in the heart regeneration of zebrafish involves the binding of Wnt ligands to Frizzled receptors on the cell surface. This leads to the activation of intracellular signaling cascades, including the canonical Wnt cascade and the non-canonical Wnt cascade (Figure 4) [88]. In the canonical Wnt cascade, the binding of Wnt ligands to Frizzled receptors leads to the activation of the intracellular protein β-catenin, which translocates to the nucleus and activates downstream target genes that promote cell proliferation and differentiation [89]. The downstream targets of β-catenin in cardiac progenitor cells include genes involved in the regulation of the cell cycle and genes involved in cardiomyocyte differentiation, such as Gata4 and Nkx2.5 [90].

In the non-canonical Wnt cascade, the binding of Wnt ligands to Frizzled receptors leads to the activation of intracellular signaling cascades, such as the Wnt/PCP cascade, which regulates cell polarity and migration [91]. Overall, this cascade plays a crucial role in the heart regeneration of zebrafish by regulating the proliferation and differentiation of cardiac progenitor cells. The activation of the cascade promotes the proliferation of cardiac progenitor cells and facilitates the differentiation of these cells into functional cardiomyocytes, leading to heart regeneration after injury [92].

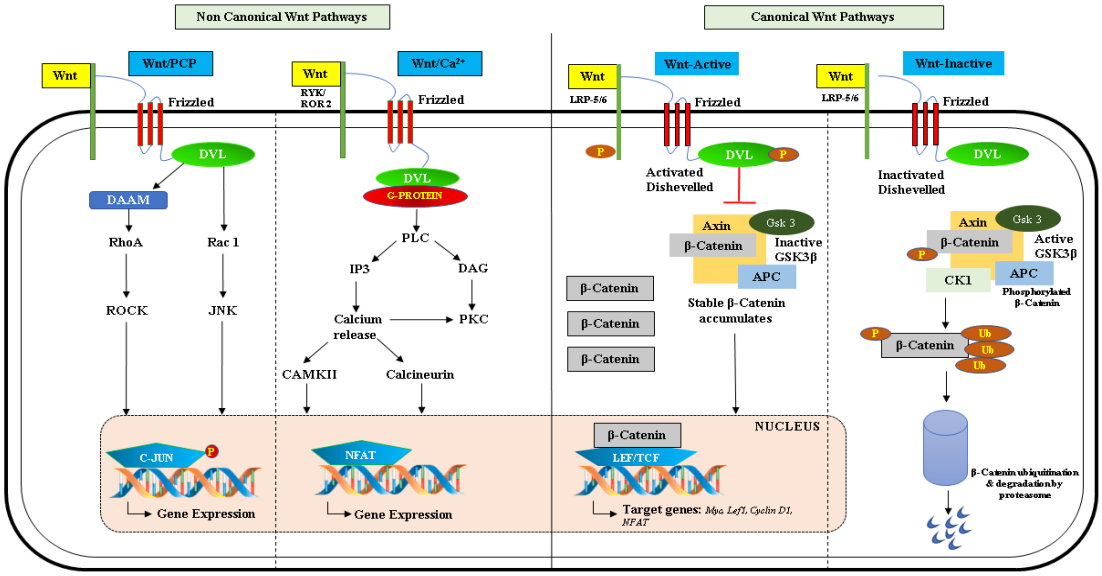

Figure (4): Canonical and Non-canonical cascade of Wnt signaling.

In canonical Wnt signaling, also known as the Wnt/β-catenin cascade, a connection between Wnt, Frizzled, and LRP-5/6 stabilizes β-catenin, which in turn regulates genes in the nucleus through LEF/TCF. Non-canonical cascades, such as Wnt/Ca^{2+}, which are triggered by Wnt-Frizzled interaction, control cellular processes independently by inducing intracellular calcium release. These cascades diverge from β-catenin participation and activate CAMKII and calcineurin for gene expression.

Activation of the FGF (fibroblast growth factor) signaling cascade

Multiple biological functions, including cell proliferation and differentiation, are regulated by the FGF signaling system. Following cardiac injury, the FGF cascade in zebrafish is activated, leading to an increase in cardiomyocyte proliferation and the development of new cardiac tissue [93]. The molecular mechanism of the FGF signaling cascade in the heart regeneration of zebrafish involves the binding of FGF ligands to FGF receptors (FGFRs) on the cell surface. This leads to the activation of downstream signaling cascades, including the MAPK/ERK cascade and the PI3K/Akt cascade [94]. In the MAPK/ERK cascade, the activation of FGF receptors leads to the activation of the downstream kinase Raf, which then activates the kinase MEK. MEK, in turn, activates the kinase ERK, which translocates to the nucleus and activates downstream target genes that promote cell proliferation and survival (Figure 5) [95]. The downstream targets of ERK in cardiac progenitor cells include genes involved in the regulation of the cell cycle and genes involved in cardiomyocyte differentiation, such as Nkx2.5 [96].

In the PI3K/Akt cascade, the activation of FGF receptors leads to the activation of the kinase PI3K, which then activates the kinase Akt [97]. Akt promotes cell survival by inhibiting apoptosis and promoting cell growth and proliferation. The downstream targets of Akt in cardiac progenitor cells include genes involved in cell survival, such as Bcl-2 [98]. Overall, this cascade plays a crucial role in the heart regeneration of zebrafish by regulating the proliferation and differentiation of cardiac progenitor cells. The activation of the cascade promotes the proliferation of cardiac progenitor cells and facilitates the differentiation of these cells into functional cardiomyocytes, leading to heart regeneration after injury.

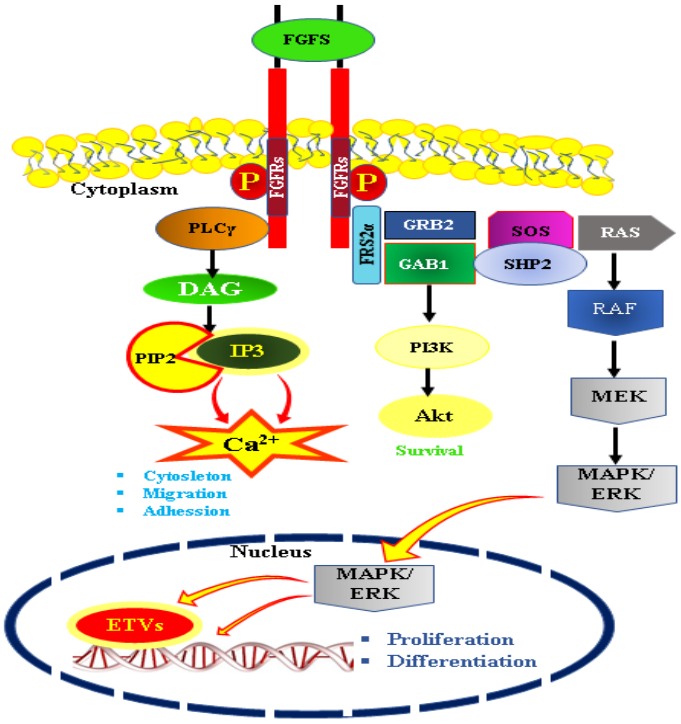

Figure (5): FGF-FGFRs signaling cascade.

Along with components like FGFS and PLCY, the FGF-FGFRs signaling cascade also integrates MAPK/ERK activation, which is crucial for cellular survival, proliferation, and differentiation. Along with affecting adhesion, cell migration, and cytoskeleton dynamics, it affects cellular behavior and function generally. When FGF ligands bind to four FGFRs, they activate cascades such as RAS/MAPK, PI3k/AKT, and PLCγ, which in turn regulate different parts of cellular functions.

Activation of the Mef2 (myocyte enhancer factor 2) transcription factor

The Mef2 transcription factor is involved in the differentiation of cardiac muscle cells [99]. In zebrafish, Mef2 is activated after heart injury and promotes the differentiation of newly formed cardiomyocytes into functional heart tissue [73]. The molecular mechanism of Mef2 in the heart regeneration of zebrafish involves its activation by various signaling cascades, including the FGF and Wnt signaling cascades. Once activated, Mef2 translocates to the nucleus and binds to specific DNA sequences in the promoter regions of target genes, regulating their expression [100]. Mef2 regulates the expression of a variety of genes involved in cardiomyocyte differentiation and maturation, including genes encoding structural proteins, such as myosin heavy chain and cardiac troponin, as well as genes involved in ion channel function and calcium handling [101]. Increasing calcium concentration in the cytosol activates calcium-calmodulin protein kinase (CaMK) which promotes the activation of Mef 2 (Mef2A, 2B, 2C, 2D) [102]. During heart regeneration in zebrafish, Mef2 is also involved in regulating the differentiation of newly formed cardiomyocytes (Figure 6). Specifically, Mef2 promotes the differentiation of cardiac progenitor cells into functional cardiomyocytes by regulating the expression of genes involved in cardiomyocyte maturation and function.

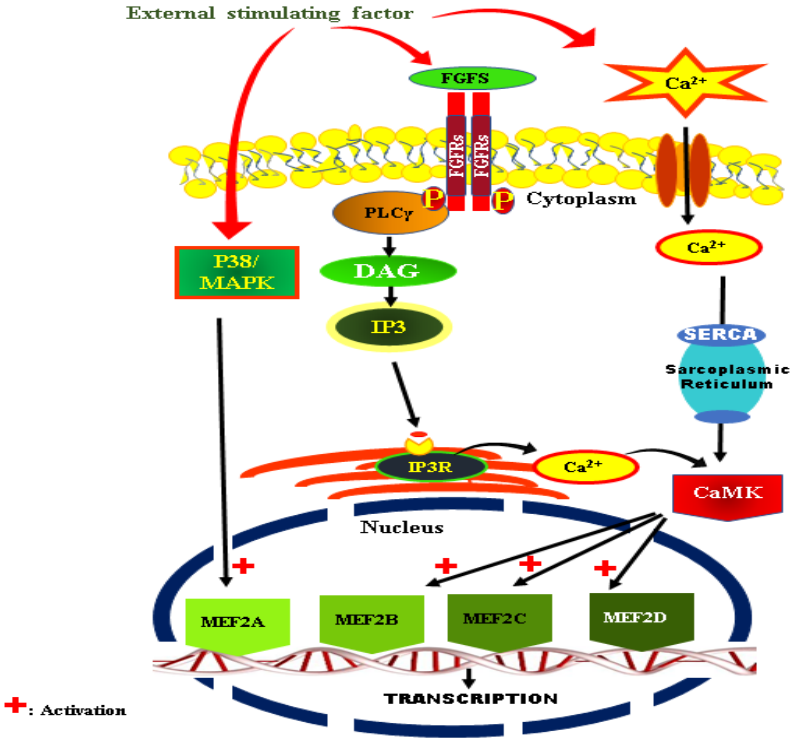

Figure (6): Mef2 signaling cascade.

External stimulation via FGF-FGFR triggers Ca^{2+} influx, activating PLCγ, P38/MAPK pathways, and releasing DAC and IP3 in the cytoplasm. Ca binds SERCA in the sarcoplasmic reticulum, activating IP3R, and allowing Ca^{2+} to enter the nucleus. CaMK activates MEF2A/B/C/D, initiating transcription in the MEF2 signaling cascade.

Inflammatory response

When a zebrafish's heart is damaged, the animal's immune system responds with an inflammatory response [103]. New cardiac tissue and the proliferation of cardiomyocytes are aided by the immune cells' production of cytokines and growth factors. The molecular mechanism of the inflammatory response in heart regeneration of zebrafish involves the activation of various signaling cascades, such as the NF-κβ cascade and the JAK/STAT cascade [104]. These cascades lead to the production of cytokines and chemokines, which attract immune cells to the site of injury [105]. In zebrafish, the recruitment of immune cells to the site of injury is mediated by the chemokine CXCL12, which is produced by epicardial cells and cardiac fibroblasts. CXCL12 activates the CXCR4 receptor on the surface of immune cells, leading to their migration to the site of injury [106]. Once at the site of injury, immune cells, such as macrophages, neutrophils, and T cells, play critical roles in the regeneration process [107]. Macrophages phagocytose debris and release growth factors and cytokines that promote tissue repair and regeneration. Neutrophils also phagocytose debris and release reactive oxygen species, which can aid in the clearance of dead cells and debris. T cells play a role in regulating the Inflammatory response and promoting tissue repair and regeneration [108]. Overall, the inflammatory response in the heart regeneration of zebrafish involves the activation of various signaling cascades, leading to the recruitment of immune cells to the site of injury, phagocytosis of debris, and release of growth factors and cytokines that promote tissue repair and regeneration. The inflammatory response is an essential component of the heart regeneration process in zebrafish and plays a critical role in promoting the repair and regeneration of damaged cardiac tissue [109].

Embracing the constraints of zebrafish

Researchers must take into account several constraints while using zebrafish, despite their significance in scientific research. A major obstacle is that they have a

duplicated genome, which makes genetic analysis more difficult, especially when compared to simpler-genome animals like nematodes or fruit flies [110]. Research dependent on antibody-based approaches is also hindered by the relative unavailability of antibodies for certain proteins, which is another problem. Limitations in researching some cardiac disorders and therapies in zebrafish compared to mammals are caused by anatomical discrepancies, such as the zebrafish heart's two chambers and lack of pulmonary circulation [111]. Zebrafish also have distinct electrophysiological and hemodynamic features, such as low central venous pressure and variations in ion currents, which affect the generalizability of results to human physiology and illness [112]. Although zebrafish and humans have certain shared cardiac characteristics, such as a similar heart rate and the length of the action potential, there are important differences in electrophysiology to keep in mind. Ultimately, zebrafish provide many benefits as a study model, but scientists using them need to comprehend their boundaries and constraints if they want their outcomes to be relevant to human health and ailments.

CONCLUSION

The zebrafish is an outstanding preclinical model with far-reaching consequences for the biological sciences, to conclude. They are priceless for investigating many genetic systems, disease pathways, and biological systems because of their human translational potential. Because of their distinct genetic and physiological characteristics, zebrafish are often used in regenerative medicine studies, especially those involving the regeneration of the heart, fins, and spinal cord. Analyzing their regeneration mechanisms and identifying important signaling cascades, such as Wnt, Hippo, and FGF, have led to the discovery of potential treatment targets. In addition, they provide a practical and economical platform for discovering new therapeutic compounds for a wide range of diseases when used in high-throughput drug screening. Zebrafish are a promising model organism for studying biology and creating cures for many human diseases because of their versatility, genetic importance, and regenerative powers. A promising avenue for future biomedical research is the creation of zebrafish illness models. These models have been very helpful in studying the etiology of various diseases, evaluating the efficacy of proposed treatments, and discovering novel avenues for treatment, including cancer, cardiovascular illness, genetic disorders, and neurological disorders. For the research of illness causes and testing of remedies, zebrafish are crucial, despite their intrinsic limitations such as a duplicated genome and physical variations. If we want zebrafish models to be more useful in pharmacological research and clinical trials, we need to find a way to get beyond these limitations.

Declarations

The authors declare that they have no conflict of interest.

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Author's contribution

Dr. Sayed Mohammed Firdous: conceptualization, supervision, and writing review & editing. **Sourav Pal, Sushmita Mohato:** writing-original draft, supervision, and writing review & editing.

Competing interests

The author has no potential confict of interest with any groups.

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Abbreviations

ADHD	Attention-Deficit/Hyperactivity Disorder
CF	cystic fibrosis
CFTR	Cystic Fibrosis Transmembrane Conductance Regulator
HD	Huntington's disease
TDP-43	TAR DNA-binding protein 43
ALS	Amyotrophic Lateral Sclerosis
TGF-β	transforming growth factor beta
VEGF	vascular endothelial growth factor
Mef2	Myocyte Enhancer Factor 2
FGF	Fibroblast Growth Factor
BMP	Bone Morphogenetic Protein
YAP	Yes-associated protein
MST1/2	Macrophage Stimulating 1
LATS1/2	Large Tumor Suppressor ½
TAZ	transcriptional co-activator with PDZ-binding motif
PCP	Planar Cell Polarity
MAPK/ERK	Mitogen-Activated Protein Kinase/Extracellular Signal-Regulated Kinase
PI3K	phosphoinositide 3-kinase
CaMK	calcium-calmodulin protein kinase
NF-κβ	nuclear factor kappa-light-chain-enhancer of activated B cells
JAK/STAT	Janus kinase/signal transducer and activator of transcription
CXCL12	C-X-C motif chemokine ligand 12
CXCR4	C-X-C chemokine receptor type 4
OI	osteogenesis imperfecta
AML	Acute myeloid leukemia
TME	Tumor microenvironment
ISVs	Intersegmental vessels
DBA	Diamond-Blackfan anemia
MDS	Myelodysplastic syndrome