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"ZEBRAFISH- A PROMISING ASPECT TOWARDS HEART REGENERATION"

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Abstract

It was long held that the adult mammalian heart was a nonregenerative, post-mitotic organ, but new research has cast doubt on this long-held doctrine. When it comes to heart regeneration responses, zebrafish may be the best-characterized model. Zebrafish are little, freshwater fish that can quickly recover from cardiac damage. When compared to human cardiovascular physiology, zebrafish's closed circulatory system and cardiac cycle are strikingly similar. After damage, the zebrafish heart can heal itself by dedifferentiating and producing more adult cardiomyocytes. Researchers at the Agharkar Research Institute in Pune, India, have successfully employed this micro-animal model to pinpoint genes involved in Formerly known as ctgfa, cellular communication network factor 2a (ccn2a) is produced in endocardial cells in wounded tissue, where it controls proliferation and repopulation of cardiac myocytes (CMs).

Key words : Ccn2a , CM proliferation , regeneration

Introduction

Zebrafsh

The zebrafish is a small freshwater fish (about 2–3 cm in length) native to the tropics and subtropics. The Indo-Gangetic plains of South Asia are home to this species of fish, and it is common to see them in paddy fields and even in still water and streams. The Danio rerio, a member of the family Cyprinidae in the phylum Cypriniformes, is a common aquarium fish native to South Asia. The striped zebrafish, which got its name because it often swims in vast schools, is a schooling fish.

Figure no.1 : The zebrafish (Danio rerio)

Table No. 1:- Scientific classificatio

Kingdom	Animalia
Phylum	Chordata
Class	Actinopterygii
Order	Cyprinifomes
Family	Cyprinidae
Subfamily	Danioninae
Genus	Danio
Species	D.rerio

Key facts

- In the 1960s, zebrafish (Danio rerio) was first used as a model organism.
- The zebrafish may be found throughout southeast Asia and is a tropical fish.
- Between 2.5 and 4 centimetres in length is typical for a zebrafish.
- Transparent as a larva, this species eventually transforms into an adult with vertical blue stripes down its length.
- Males are often pink or yellow in colour and have the appearance of a torpedo.
- The eggs that a female carries make her less pink and give her more body fat than a male.
- Zebrafish are a valuable model for studying the mechanics of development and disorders like cancer, and have already been used to shed light on some of the basic processes underlying muscular dystrophy.
- In 2013, scientists released the whole sequence of the zebrafish genome.

Benefits of the zebrafish

- Zebrafish is small and robust.
- Costing less to care for than mice, for example.
- The onset of daylight causes sexual activity in zebrafish (many other fish only lay eggs in the dark).
- Each zebrafish female can generate hundreds of fertile eggs, giving researchers a steady stream of new embryos to examine.
- Their pace of development is so rapid that in a single day they progress as much as a human embryo does in a full month.
- Transparent zebrafish embryos make it easy for scientists to study how organs and tissues form. Using a simple microscope, researchers were able to observe every single blood vessel in a developing zebrafish. Because zebrafish embryos are fertilised and grow outside the mother's body, they are a great experimental subject for researching development in the womb.
- There are many similarities between the genomes of zebrafish and humans. They are 70% genetically similar to us.

- 84% of human disease-linked genes are conserved in the common aquarium fish, the zebrafish.
- The zebrafish is a vertebrate, thus it shares many of our key body parts and tissues with other vertebrates like us. They have many human-like characteristics in their skeletal and muscular systems, as well as their blood, kidneys, and eyeballs.
- Zebrafish have the extraordinary capacity to mend damaged heart muscle. To give just one example, if a portion of their heart were to be destroyed, it might regenerate in a couple of weeks. Scientists are currently investigating the underlying mechanisms of this process in the hopes that it may lead to the creation of treatments for heart damage in humans.
- • A complete, high-quality sequence of the zebrafish genome has been completed. Due of this, scientists have been able to develop. To investigate the role of over 14,000 genes.

History of zebrafish

The University of Oregon scientist and tropical fish fanatic George Streisinger was active in this field in the 1960s.

All stages of a zebrafish embryo's development can be examined and controlled because it is created in a petri dish rather than the fish's body.

George wanted to research the structure and function of the nervous system, but he wanted to do so using a vertebrate model that was physiologically simpler than the mouse. George and other Oregon scientists published many seminal studies on the zebrafish in the early 1980s, which garnered international attention.

The zebrafish appears to combine the best aspects of all the other models, making it close to ideal as a model organism for vertebrate development. Zebrafish embryos are similar to frog embryos. Grow in a way that allows them to be observed and influenced as they go. Yet in contrast to the frog, the embryo is less complex, grows at a more rapid rate, and, like those of worms and fruit flies, is see-through.

The zebrafish, like the mouse, can be utilised in genetic studies because of its two- to three-month generation cycle. In contrast to the mouse, the zebrafish has a higher reproductive rate and a smaller body size (200 eggs per week compared to a mouse litter of 15 pups in 21 days).

The Wellcome Trust Sanger Institute initiated the zebrafish genome project in 2001 and finished it in 2013. More than 26,000 protein-coding genes were discovered when the zebrafish genome was sequenced. This is the biggest number of protein-coding genes discovered in any vertebrate. It also demonstrated that 70% of human genes are conserved in at least one zebrafish gene.

Development of zebrafish

The zebrafish has an extremely rapid rate of development; by 24 hours post fertilisation (hpf), the fertilised egg has evolved into an embryo that has established most organ primordia, including a developing nervous system and a heart tube that is beginning to contract. After this, the heart begins to beat and blood begins to circulate throughout the body. After around 2 days following fertilisation (dpf), the embryo hatches, and by 3 dpf, most of its internal organs have developed to the larval stage. Zebrafish begin their metamorphosis at 30 dpf and achieve adulthood after 90 days.

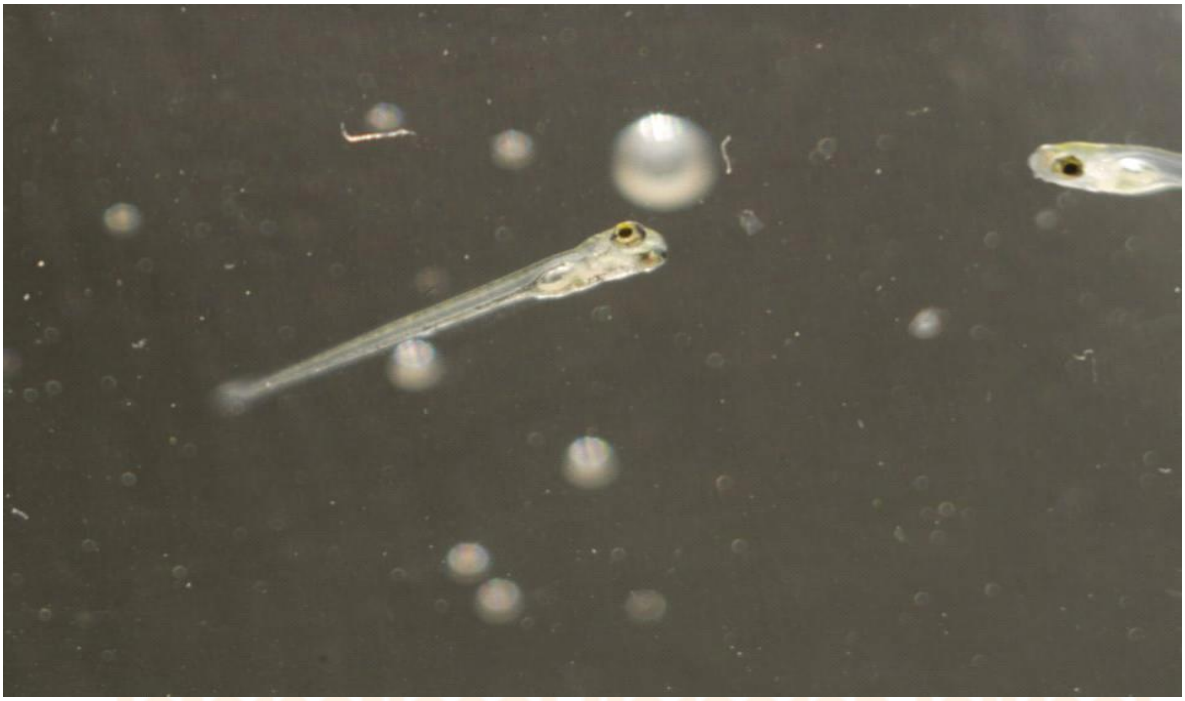

Figure no. 2 : Zebrafish larva : (at the developmental stage between 3-30 days)

ZEBRAFISH : a suitable research model

The Indo-Gangetic plains of South Asia are home to this species of fish, and it is common to see them in paddy fields and even in still water and streams. Adulthood occurs after three months, and the fish can live for two to three years in captivity.

However, zebrafish have become a significant model for human disease because 70% of protein-coding human genes are related to genes found in zebrafish and 84% of disease-associated genes have a zebrafish equivalent.

The transparency of this fish throughout its embryonic stages is one of its distinguishing features. This transparency makes it possible to see the developing fish's internal organs, including its beating heart and blood vessels. Researchers in developmental biology are drawn to the zebrafish because of its remarkable ability to repair nearly

every part of its body. Because of this, biomedical scientists can use it as a research model. Studying human neurological, endocrine, and cardiovascular problems, as well as medication discovery and toxicity testing, can all benefit greatly from using zebrafish as a translational model.

There some specific traits of zebrafish that make it a suitable model for studies :

- **Low cost maintenance**

  Due to the zebrafish's small size and uncomplicated natural environment, up to 40 individuals can be maintained in a tank no bigger than a regular mouse cage. Furthermore, the overall budget is reduced because lower amounts of pricey chemicals are employed in drug development trials.

- **Size**

  The small size (about two to three centimetres) of zebrafish allows for straightforward examination of organ systems or their possible use in transplantation. While its large size is an asset in many processes, it may not be a sufficient sample for some types of analysis.

- **Fecundity**

  Due to their huge clutch size (200-300 eggs per female zebrafish), zebrafish are ideal for studying uncommon genetic incidents and testing parallel theories. The short time it takes for these fish to reach adulthood is also a boon to researchers.

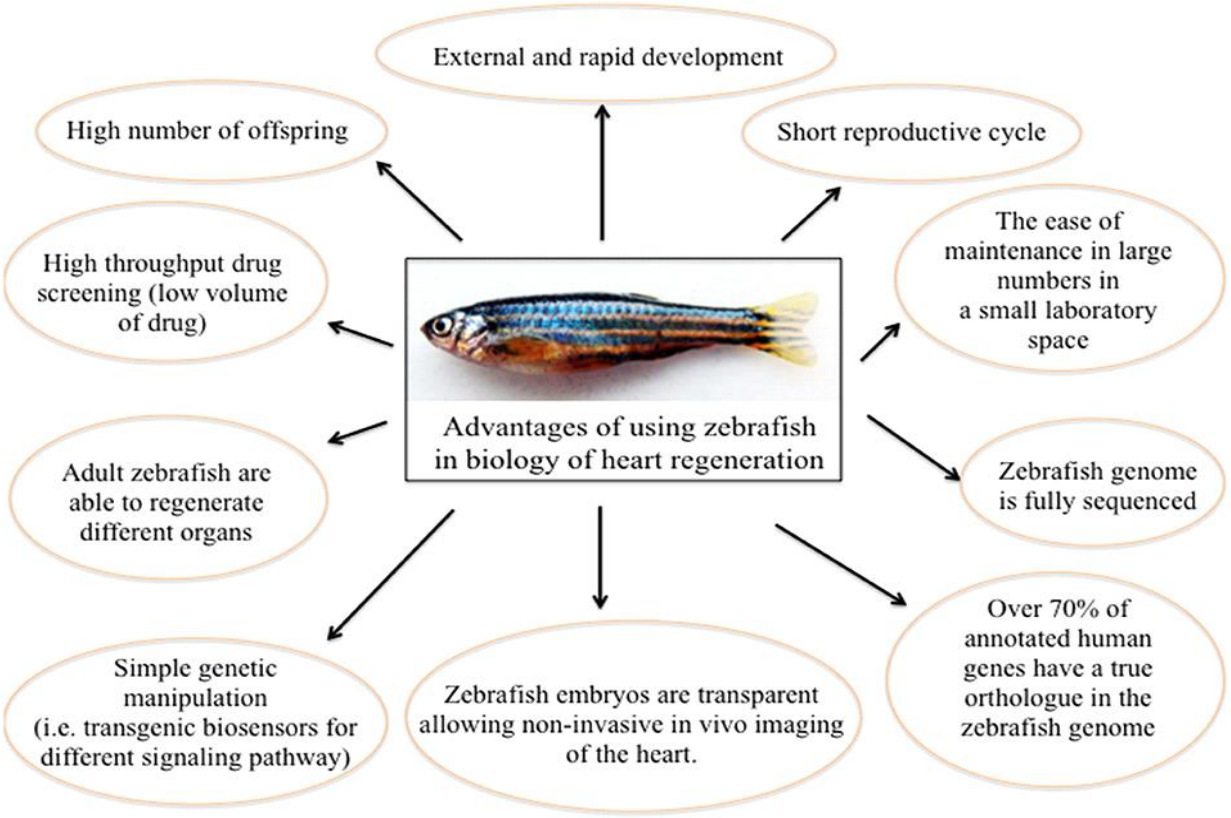

Figure no 3 : The significance of using zebrafish

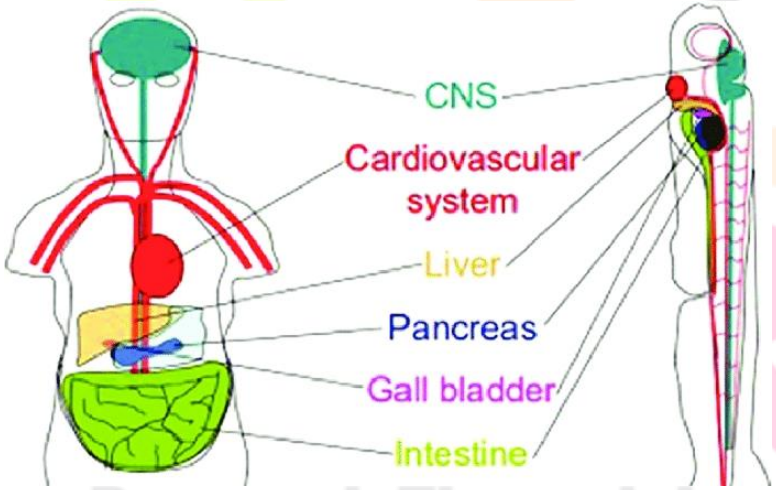

Figure no.4 : conserved organ systems between human and zebrafsh

Conserved organ between human and fish

Humans and zebrafish may at first glance seem very dissimilar, yet there are actually many similarities between the two. Zebrafish actually share 70% of the human gene pool.

Zebrafish also feature two eyes, a mouth, brain, spinal cord, gut, pancreas, liver, bile ducts, kidney, oesophagus, heart, ear, nose, muscle, blood, bone, cartilage, and teeth. The genes and crucial pathways necessary for the development of these characteristics are remarkably similar in humans and zebrafish. Therefore, it should be possible to model in zebrafish any disease that alters these elements of the human body.

Why use zebrafish when we could use mice

Zebrafish have various benefits over their hairy counterparts, despite the fact that mice are more closely related to humans evolutionary speaking due to the fact that they are mammals. Zebrafish are beneficial since the adults are small and tend to live in huge groups called "shoals." Therefore, they take up far less room and are easier to care for than mice. Additionally, mature zebrafish can lay anywhere from 50 to 300 eggs at a time, and they reproduce often (about every 10 days). Contrast this with mice, which typically have litters of between one and ten pups and can have no more than three litters in their whole lives. The results of scientific tests must often be replicated numerous times to establish their reliability; therefore, it is useful to have access to an animal that can repeatedly reproduce in huge numbers.

Additionally, zebrafish embryos are placed and fertilised externally, making them amenable to a wide range of manipulations. It is possible to perform in vitro fertilisation if it becomes essential. Transgenic or knock-out zebrafish lines can be easily created by injecting one-cell-stage fertilised eggs with DNA or RNA to permanently alter their genetic makeup. This type of work with mice is far more difficult. In order to access and alter mouse embryos, the mother mouse must be killed. They would need to be put into another female mouse following fertilisation or injection to ensure their survival.

In addition, the transparency of zebrafish embryos makes it possible for researchers to observe the development of fertilised eggs all the way through to newborn fish. Transgenic zebrafish embryos can have their fluorescently tagged tissues seen through their clear bodies. Since mouse embryos are not transparent and grow inside their mothers, it is not possible to observe their growth in real time like it is in zebrafish.

There is a cap on the disorders that may be examined with zebrafish, though. A separate animal model is necessary for studying human diseases caused by genes that are absent in zebrafish. To add insult to injury, zebrafish are poor models for human diseases that manifest primarily in a human-specific tissue or body component (e.g., prostate, mammary glands, lungs).

Ability to heal their heart

Myocardial wall in zebrafish is made up of myocardium, epicardium, and endocardium, same like in mammals; there are only two chambers in a mammal's heart. The myocardial of zebrafish, like that of humans, has compact and trabecular layers. Numerous embryonic investigations and forward genetic screens have shown that the zebrafish is a valuable model for studying the human heart and human disease. The regenerative capacity of the zebrafish heart suggests a strategy for lowering the morbidity and mortality rates associated with cardiac disorders, including MI.

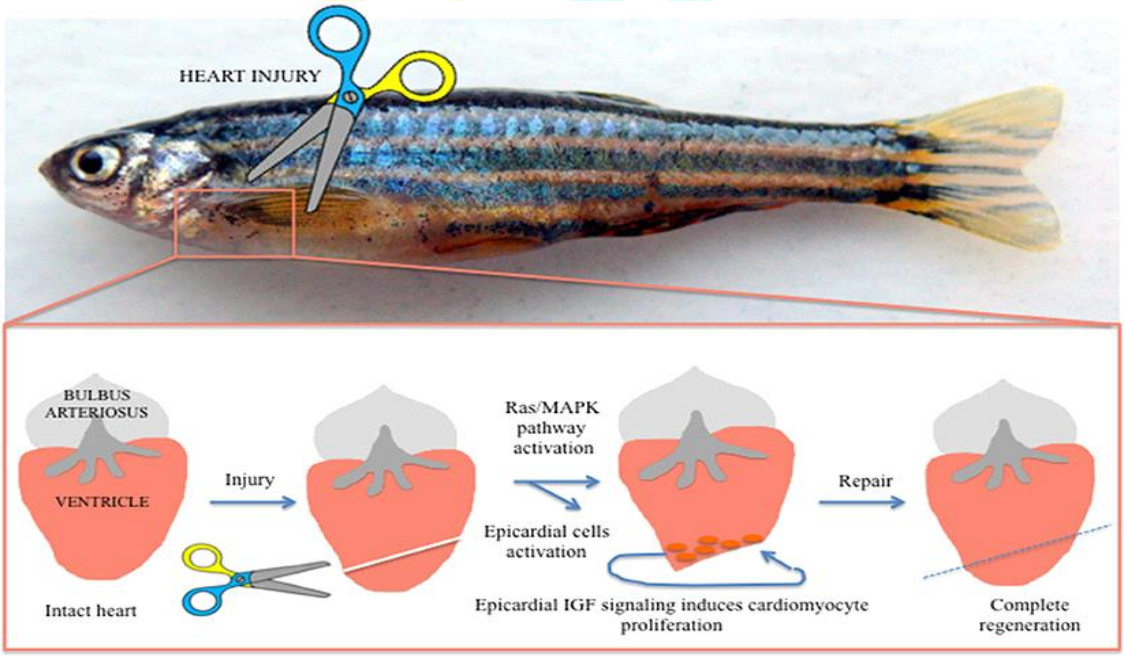

Figure no.5: Phases of healing/regeneration of hear

Gene that promotes heart regeneration

This little fish served as a model for the identification of genes and proteins that stimulate heart regeneration. They put in a lot of study and finally found the gene responsible for promoting heart regeneration by increasing cardiomyocyte proliferation: cellular communication network factor 2a (ccn2a).

Dr. Chinmoy Patra, Head of the Max-Planck Partner Group, Department of Developmental Biology, Agharkar Research Institute scientists Dr. Patra, Debanjan Mukherjee, and others, had emphasised the importance of using zebrafish as a model organism, saying, "Ccn2a promotes the innate regenerative response of the adult zebrafish heart and maybe a promising therapeutic target for humans." The gene that aids in heart regeneration has now been located.

Increased expression of this gene promotes the growth of cardiomyocytes, the cells responsible for contracting the heart muscle. Researchers have also found that this gene can speed up the healing process by removing the temporary collagenous fibrotic scar.

Ccn2a

In zebrafish, Ccn2a is an injury-induced matricellular factor that stimulates the growth of new cardiac myocytes in response to mechanical damage. Endocardial cells in damaged tissue activate cellular communication network factor 2a (ccn2a), formerly known as ctgfa, which controls CM proliferation and repopulation.

The adult zebrafish heart's inherent regeneration response is positively regulated by Ccn2a. In contrast to mammals, adult zebrafish hearts can repair themselves if a section of myocardium is removed. Following the removal of 20% of the myocardium, a fibrin-rich clot forms, which is then replaced by new cardiomyocytes rather than fibroblasts. These new cells differentiate from cardiac progenitor cells in a process determined by the epicardium, which appears to recapitulate many elements of embryonic cardiac development.

A mutation in the cell cycle regulator Mps1 restricts proliferation in zebrafish, making them act like humans after injury. This results in scar formation. If the heart of a zebrafish can be coaxed to regenerate, it's exciting to think that maybe it might be done in a human.

- **The CCN gene family comprises six members formerly known as**
- cysteine-rich angiogenic inducer 61 (CYR61, now CCN1),
- connective tissue growth factor (CTGF/CCN2),
- nephroblastoma-overexpressed (NOV/CCN3),
- WNT1-inducible signaling pathway 1 (WISP1/CCN4),
- WNT1-inducible signaling pathway 2 (WISP2/CCN5), and
- WNT1-inducible signaling pathway 3 (WISP3/CCN6).
- The proteins in this family all include the same tetramodular domain and play important roles in development and physiology by controlling things like cell adhesion, migration, proliferation, differentiation, and survival.

In zebrafish, the proliferation of existing cardiomyocytes rather than the differentiation of stem cells or other cells is responsible for cardiomyocyte regeneration. Therefore, further research into the heart regenerative mechanisms of the zebrafish may lead to the identification of particular molecules able to govern the proliferation of preexisting cardiomyocytes.

Cardiomyocyte proliferation

Cardiomyocyte proliferation refers to the process by which cardiac muscles undergo regeneration through proliferation.

The mammalian cardiac system develops from the first and second heart fields. Precursor cells from the heart field move toward the embryonic centre and unite there to form the first linear heart tube. The cardiomyocyte precursors are added to the poles, and the heart tube grows longer. Proliferation of contracting cardiomyocytes then "balloons" the chambers and causes the heart to enlarge. Mammalian cardiomyocytes cease replicating and entering the cell cycle shortly after birth.

There are currently no medicines that can effectively reverse heart damage and remodelling, and for a sizable subset of patients, further decline is not preventable. Therefore, the research and development of new treatments is critically needed.

Newborn mice and zebrafish, in contrast to adult mammals, are able to heal their hearts following experimentally produced injury by inducing cardiomyocyte growth. Therefore, a lot of work has gone into trying to figure out if it's possible to stimulate the growth of cardiomyocytes in adult mammals.

There has been a growing amount of research in recent years showing that stimulating cardiomyocyte proliferation may one day be used to stop the decline in heart function that occurs after injury and even reverse existing damage.

Human diseases that have been successfully modeled in zebrafish

Evidence suggests that Duchenne muscular dystrophy in humans can be modelled in zebrafish by knocking out the dystrophin gene.

Patients with Duchenne muscular dystrophy carry mutations in dystrophin and show increasing muscle weakening beginning in childhood. The absence of dystrophin causes necrotic muscle fibres, which are then replaced by inflammatory cells, fibrosis, and muscle fibres of aberrant sizes in both humans and the zebrafish model.

Promising therapeutic target for human

Unlike the skin and the liver, the human heart is not regenerative. Myocardial injury cannot be repaired in humans, and the damaged heart muscle of a person who has had a heart attack cannot be functionally healed, leading to impaired pumping ability. As an alternative, this one-of-a-kind fish has the ability to repair its damaged heart and return to normal functioning.

Humans have not had access to a treatment that can repair heart damage. Therefore, researchers have used this animal as a model because it can regenerate its heart so effectively on its own, and they hope that by learning how it does it they might develop techniques to facilitate heart regeneration in humans. Industrial researchers frequently use this paradigm. The zebrafish research facility was built by Dr. Reddy's lab in Hyderabad.

Drawbacks and limitations

While zebrafish have been useful in studying other diseases, such as cardiovascular illness, there are greater challenges when utilising them to study metabolic disease.

Lifestyle, behaviour, and socioeconomic characteristics unique to humans that influence metabolism cannot be recreated in zebrafish.

Since zebrafish growth depends on numerous unpredictable elements like temperature, body fat, heredity, etc., tracking their diet individually is also a challenge.

Blood sample is time-consuming and difficult to repeat in zebrafish, making it difficult to undertake metabolic assays such as hormone testing and insulin tolerance tests.

Intraperitoneal injections, for example, are more difficult to execute in zebrafish than in other animal models, such as mice.

Future Perspectives

The zebrafish's journey as a model organism in cardiovascular research has been incredibly fast-paced and fruitful. Zebrafish will continue to be utilised as an inexpensive in vivo model to evaluate candidate genes (identified by microarray or genome wide association studies) that may be associated with a cardiac trait.

Few areas in cardiovascular research have not yet put the zebrafish model to the test, but that is quickly changing. The zebrafish community has a formidable challenge: improving the accuracy with which they reproduce human cardiovascular dysfunction in their model organism.

The ability to create mutants for every gene has been greatly expanded by recent technological advances. As a result, mutants for practically every gene will certainly become available in the not-too-distant future. This makes it much simpler and more cost-effective than studying mice to examine genetic connections between genes, gene-environment interactions, or the involvement of modifier genes, all of which are crucial drivers of the phenotype severity of a given mutation.

Exciting developments have been made in zebrafish, which are similar to mice in that they may be genetically manipulated using increasingly sophisticated methods. Although the zebrafish cannot totally replace the mouse, it will help cut down on the amount of animal tests. Furthermore, the zebrafish model will be particularly useful in the context of the latest developments in mass screening technologies like chemical genetics.

WHO reports on cardiovascular disease

At an annual rate of 17.9 million, cardiovascular diseases (CVDs) constitute the leading cause of death worldwide. Coronary heart disease, cerebrovascular illness, rheumatic heart disease, and other ailments all fall under the umbrella of cardiovascular disease (CVD). Heart disease and cerebrovascular disease are responsible for four out of every five fatalities from CVD, and one-third of those deaths occur in those younger than 70.

High-risk individuals may be overweight or obese, have hypertension, diabetes, or abnormal lipid profiles. These are all readily measurable in primary care settings. It is possible to reduce the number of premature deaths caused by CVDs by identifying people at highest risk and ensuring they receive treatment.

Key facts

- Cardiovascular diseases kill more individuals each year than any other group everywhere in the world.

- It is projected that in 2016, CVDs were responsible for the deaths of 17.9 million individuals worldwide, or 31% of all deaths. Heart disease and cerebrovascular accidents account for 85% of these fatalities.

- More than seven out of ten people who lose their lives to cardiovascular disease live in nations with poor or moderate per capita income.

- In 2015, 17 million people died younger than 70 from noncommunicable diseases; of these, 82% occurred in low- and middle-income countries; and 37% were attributable to cardiovascular disorders.

- Tobacco use, poor nutrition leading to obesity, lack of exercise, and excessive alcohol use are only few of the preventable behavioural risk factors for cardiovascular disease that can be addressed through population-wide programmes.

- Early detection and management, via counselling and medicines as appropriate, is necessary for people with cardiovascular disease or who are at high cardiovascular risk (due to the presence of one or more risk factors such as hypertension, diabetes, hyperlipidaemia, or already established disease).

India with cardiovascular disease

Nearly a quarter (24.8%) of all fatalities in India are due to CVD, according to age-standardized estimates from the Global Burden of Disease research in 2010. India has a higher rate of death from cardiovascular disease (CVD) than the rest of the world, with 272 deaths per 100,000 people when adjusted for age.

Estimates of the Global Burden of Disease are derived from smaller, community-based studies, and there is a large information gap, especially in regards to the factors that lead to death in rural India. Verbal autopsy data were obtained in India after 2004, but they were not factored into the burden estimate since they were neither examined nor made available to the public.

The prevalence of cardiovascular disease and the long-term trends in cardiovascular mortality in India are not known due to a lack of nationally representative surveillance data. While the Global Burden of Disease study reported a rate of CVD-related deaths of 255 per 100,000 males and 229 per 100,000 women, three recent reports of major prospective studies in India suggest a larger proportion of mortality owing to CVD (30-42%). Major causes of death in India are ischemic heart disease (IHD) and stroke, with IHD being the more common of the two. India has a death rate from IHD that is on par with that of Western industrialised countries, which is much higher than the global norm. Overall, IHD and stroke account for almost 21.1% of fatalities and 10% of lost life expectancy in India (years of life lost is a measure that quantifies premature mortality by weighting younger deaths more than older deaths). Years of life lost due to cardiovascular disease in India rose by 59% between 1990 and 2010. (23.2 million to 37 million).

Summary

Although no animal can serve as a perfect model for a human disease, the benefits of the Zebrafsh model for cardiac regeneration research are laid out in this study. In particular, we learn that the recovery of CM number is critical to cardiac regeneration and the return to full functional capacity. Some of the first medications identified in zebrafish are now in clinical trials, further demonstrating the translational value of this model, and studies of various zebrafish disease models have proved the high relevance of this approach. Researchers think these little striped fish could one day lead to major breakthroughs in the field of medicine.

Conclusion

Unfortunately, many human organs and tissues are incapable of regeneration. As a result, an external stimulus is required to initiate regeneration activity in human tissues.

Myocardial infarction (MI) causes irreparable damage to heart tissue in humans, leading to higher rates of morbidity and mortality. Danio rerio (zebrafish) have a complete regenerative response that allows them to mend broken and injured hearts. Molecular processes that can elicit and maintain a regenerative response after injury can be better understood by examining model species that can recover lost cardiac tissue after injury, such as the zebrafish. These findings hold promise for the development of novel therapeutics for treating and preventing heart attacks in humans by eliciting a more effective regeneration response.

The zebrafish model will provide complementary data to other models and provide important new insights into heart regeneration, with the ultimate goal of developing effective treatments for heart failure.

In conclusion, the future of the zebrafish model is secure with well-designed, careful experiments.

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