REVIEW

A Review on Zebra Fish as an Experimental Model for Studying Craniofacial Development Disorders.

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Abstract

Zebra fish (Diano riro) is becoming a popular animal for studying human developments and diseases. This is animal has become widely accepted due to its genetic and physiologic similarity to humans. More than 70% of human genomes' orthologus genes can be found in this animal. The embryos are formed outside the animal's body and are translucent, so that the embryological development can be visualized using a low power microscope. The cranial bone development and suture formations of zebra fish is similar to human beings. Molecular studies have found that genetic and protein expressions during cranial development of zebra fishes are similar to humans. Transgenic zebra fishes were also utilized to study cranial disease conditions like craniosynostosis. Zebra fish as an animal model has a promising future in craniofacial development research.

Key words: Zebra fish, transparent embryonic development, craniofacial.

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Introduction

Animal experiments are important in biomedical research. Animal experimental models are developed in order to understand a disease or bodies response to the exposure of toxins or drugs, so that human beings are spared of any harmful effects. Usually the animal experiments are conducted in mammals. But practical difficulties like cost and time involved and the regulatory constraints due to animal ethical concerns have made researchers to look for other alternatives.

Alternative animal experimental models have been developed using invertebrates and lower vertebrates like fishes in order to overcome these difficulties. In recent years Zebra fish *(Danio rerio)* is being frequently used as an experimental model for biomedical research.[1]

Zebra fish is a small tropical fresh water fish seen in the Ganges river in eastern and in north India. They are commonly found in slow moving and stagnant waters. In their natural habitat they are usually bottom dwellers.[2] These fishes are characterised by black longitudinal stripes. Males are slender in shape while females are fatter in order to carry eggs (Fig 1).[1,2]

Why Zebra fish is a popular vertebrate animal model?

Economy and maintenance: These fish are easy and cheaper to maintain than laboratory mice. Relatively small space is only required to maintain large numbers of fishes. It reproduces at a faster rate producing 200 to 300 eggs daily so that enough progenies are produced in a short span of time. Their maturation time takes only 90 days time.[3]

Homology to human genome: The complete genome sequence of zebra fish was sequenced and published in 2013. When zebra fish genome was compared with human genome it was found that 70% of human genes had zebra fish gene analogues with similar functions and 84% of genes associated with human diseases have zebra fish gene analogues.[4] This makes zebra fishes a good animal model to study the human diseases at the genetic level.

Transgenic zebra fish models have been developed by knocking out or knocking in specific genes. This creates an opportunity to study the developmental and physiological effects of the gene alteration on the organism which can be translated to human diseases.[5]

Similarity to human organ physiology: Zebra fish have same major organs and tissues like the humans. It was also found that Zebra fishes have similar toxicity tolerance and circadian rhythms like mammalian models. They also have the ability to regenerate tissue of heart, photoreceptor cells, retinal neurons and spinal cord. The muscle, blood, kidney and eyes share many features with the human tissues.[2]

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Translucent embryos: In zebra fish as fertilization and development of embryos occur outside the body of the mother. The embryos of zebra fish are translucent and the stages of embryological development can be monitored by the researcher with a low powered microscope. The zebra fish embryo forms the complete organ systems by 48 hours after fertilization. They complete the embryogenesis in 72 hours. This makes Zebra fish an excellent animal model for studying embryological development and teratogenic effects of various chemicals. [1,5]

Ethical concern: The standard operating procedure set by Committee for the Purpose of Control and Supervision's (CPCS) for institutional animal ethics committee has named non-vertebrates like Drosophila melanogaster, Caenorhabditis elegans and lower vertebrates like zebra fish as alternatives of animal studies from ethical perspective. They have recommended its use as a replacement to animal experiments where ever possible.[6,7]

Zebra fish as an animal model for craniofacial development

Zebra fishes show significant genetic and anatomic homology with humans. Studies by Longaker *et al* showed that anatomy of zebra fish skull vault and cranium is similar to mammals. The cranial vault of zebra fish is composed of frontal parietal and supra occipital bones. Like mammalian skull they found that zebra fishes also have interfrontal, sagittal, coronal and lambdoid sutures. The cranial sutures were found to be similar to mouse cranial sutures. Like mouse cranial sutures the Zebra fish cranial sutures also remain open or patent.[8] Studies by Topczewska *et al* and Longaker *et al* demonstrated that like the human skull bones zebra fish skull bones are also formed by intramembranous ossification when differential staining for calcium and cartilage.[8,9]

Longaker *et al* found that in zebra fishes, the first signs of osteogenesis were detected at the 27-day post fertilization (dpf) starting from the frontal bone. By 51 dpf mature frontal parietal and occipital bones were seen.[8] Shoela et al has shown that that the cranial growth of the zebra fish is strongly correlated with the skeletal development as indicated by the standard length and they found that the cranial suture development is analogous to mammals.[10] Studies have also shown that the connective tissue in the sutural areas of zebra fish skull expresses genes needed for suture development which were similar to mammalian. [9]

In mammals' cranial sutures remain open to allow the growth of the cranial bone and brain. Accelerated growth and fusion of the cranial bones lead to craniosynostosis disorders in humans. This condition is seen in 1 in every 2500 live births. Craniosynostosis will lead to restriction in brain growth, increase in intracranial pressure and other developmental impairments. [9] Teng et al studied genetic causes of Saethere-Chotzen syndrome which is the second most form of craniosynostosis, where coronal suture prematurely closes. This synostosis is attributed to the loss of function mutation of TWIST1 gene which codes for helix loop helix transcription factors. Teng et al was able to develop a transgenic zebra fish model where they knocked out the homologous gene of TWIST1 gene. They found that with the loss of function of the TWIST1 gene resulted in the fusion of coronal suture of zebra fish cranium which otherwise in normal zebra fishes remain patent.[11] These studies show that zebra fish can be used as an animal model for studying the human cranial development in diseased states.

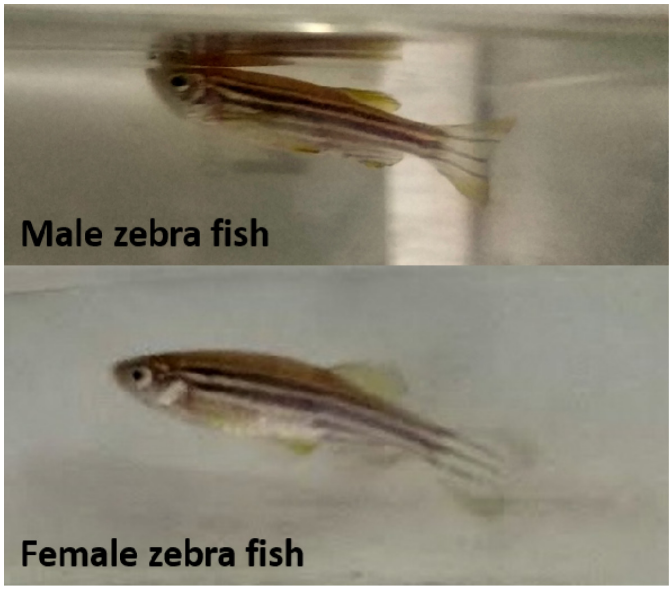

Figure 1: Male and Female Zebra Fishes

Zebra fish as an animal model for other human experimental studies

Cancer research: When zebra fishes were exposed to carcinogenic agents, it was found that they developed cancers. Various studies have also been done by tumour transplantation in zebra fish. Using these models' researchers were able to study tumour characteristics like tumour cell migration, metastasis, angiogenesis and response to therapeutic agents. Various important genes and pathways are found to be similar in humans and zebra fish.[2]

Hematopoietic research: Zebra fishes were found to have similar multilineage hematopoietic process like human beings. So many scientists have used zebra fishes to study hematopoietic pathways and blood disorders.[1]

Cardiovascular diseases: The external embryological development of zebra fish allows sequential development of heart and the vascular system. Wild and transgenic zebra fishes have been used to study defects in the size, shape and functions of heart. Since zebrafishes have the ability to regenerate cardiac tissue, they have also been investigated for cardiac stem cells.[1,2]

Zebra fish in diabetic research: Zebra fish is now used as an alternative model for studying diabetes and treatment. When the zebra fish was exposed to high glucose levels it was found to become hyperglycaemic and even found to develop retinopathies.[2]

Neurodegenerative diseases: The brain of zebra fishes is similar in its organization with human brain as fore, mid and hind brain and have many similar signalling proteins. Homologous genes and biochemical pathways for neurological disease like parkinsonism, Huntington's disease and Alzheimer's disease were found in zebrafish making it valuable research tool.[2]

Behavioural studies: Zebra fishes display complex behaviours like memory and conditioned responses. Zebra fish is now used as an animal model for studying depressive disorders. Behavioural tests of zebra fish for cognitive, approach avoidance and social paradigms are now available. It is possible to study the behavioural responses of zebra fishes to physiological, environmental and pharmacological alterations.[3]

Zebra fishes for drug screening: Zebra fish is being used as low-

cost screening in the pre regulatory phases of drug development. The zebra fish larva is exposed to drugs at various concentrations in multi well plates. They are evaluated for effects of toxicity.[2]

Conclusion

Zebra fish is evolved as a popular low-cost animal model for studying various disease process. Formation and development of embryos outside the body, translucency of embryos and similarities to human development makes this animal mode ideal for studying developmental disorders. The use of this model in studying the craniofacial embryological development and the associated disorders is promising. Using the zebra fish model to study the effects of drugs and environmental pollutants on the craniofacial skeletal development has great future research potential.

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