International Journal of Pharmacy and Biological Sciences-IJPBS™ (2019) 9 (2):902-907
Online ISSN: 2230-7605, Print ISSN: 2321-3272
Research Article | Biological Sciences | Open Access | MCI Approved
UGC Approved Journal

Zebra Fish as a Model for testing the Efficacy of Herbal Medicine

Palinchandra Sharma Aribam[1], Karuna Devi Longjam[2], Kishan Kshetrimayum[3], Usharani Longjam[4], Archana NG[5], Santosh Keisam[6] and Premila Chanu Oinam[7]*

[1, 2, 3, 4, 6, 7]Advanced Level Institutional Biotech Hub, Modern College, Imphal-795010, India.
[5]Regional Institute of Medical Sciences, Imphal-795004, India.
[7]Department of Zoology, Modern College, Imphal-795010, India.

Received: 20 Jan 2019 / Accepted: 12 Mar 2019 / Published online: 1 Apr 2019
Corresponding Author Email: opchanu@gmail.com

Abstract

The Zebra fish (*Danio rerio*) is an ideal model organism for the study of vertebrate development since the 1960s. Zebrafish is a tropical freshwater fish found in the rivers and ponds of India belonging to the *Cyprinidae* family of the order *Cypriniformes* and native to Himalayan region. It is commonly kept in aquaria in India. Zebra fish have a similar genetic structure to humans with around 70% similarity and similar major organs and tissues as humans. It is an important model for biomedical research, drug development, diseases, toxicological studies, clinical therapy etc.

Keywords

Zebra fish, liver disease, diabetes, kidney disease, herbal medicine.

*****

INTRODUCTION:

The zebra fish (*Danio rerio*) is a tropical freshwater fish of Himalayan region and commonly kept in aquaria in India and is found in slow-moving or stagnant water. It belongs to the *Cyprinidae* family of the *Cypriniformes* order [1,2,3,4]. It is an important and widely used vertebrate model organism in research work due to large fecundity and fertility rate as well as regenerative abilities and has been genetically modified by researchers to produce many transgenic strains, can be kept easily cheaply in large numbers in laboratory where it breeds all-round the years and economic screening during per regulatory phases and study of toxicity very easily. Zebra fish as an experimental model is increasingly used in molecular research for gaining popularity in the fields of biomedical research and toxicology nowadays [5,6,7,8]. As a vertebrate model, zebrafish possess several advantages such as a very rapid embryonic development outside the mother`s body and well characterized behavior of easy observation and they have a fully genome with 400 distinct genes and greater than 2000 micro satellite markers are similar to human genome (about 75%) that gives the exact idea for many genes which are involved in human diseases can be matched with zebra fish genome very effectively. Different strains of drugs such as tetracycline, penicillin, herbal drugs, vaccine etc have been successfully experimented in zebra fish [9,10,11,12,13].

Importance of zebra fish in scientific research

Danio rerio is useful scientific model for studying vertebrate development and gene function. It was first use as laboratory model by the American

molecular biologist George Streisinger, University of Oregon 1970s-8s [1]. Zebra fish are omnivorous, primarily eating zooplankton, insects, insect's larva and phytoplankton. There are five uniform, pigmented horizontal blue strips on the side of the body which are similar to zebra`s stripes and extend to the end of the caudal fin so it is given name as zebrafish. Male fish are slightly smaller than female, torpedo shaped with gold stripes between the blue stripes. Female fish has whitish belly silver stripes instead of gold. They can grow up to 6.4cm (2.5in) in length, although it seldom grows larger than 4cm (1.6in) and lifespan is around two to three years although in an ideal condition this may be extended to over five year [2,5,14].

Adult females are able to lay 200-300 eggs in each clutch. Upon release, embryonic development begins, growth stops after the first few cell divisions. Fertilized eggs are transparent that makes zebra fish a convenient research specimen. Development progress are very rapidly, and development of all major organs starts within 36 hours of fertilization and hatching takes place 12-36 hours later depending on the embryo`s internal and external condition. Swimming and feeding behavior begin about 36 hours later [15]. Scientific experiments are generally repeated multiple times in order to get the accuracy of results and having specimens with large number of offspring is helpful to the experiment. Zebra fish have two eyes, a mouth, brain, spinalchord, intestine, pancreas, liver, bile ducts, kidneys, oesophagus, heart, ear, nose, muscle, blood, bone, cartilage and teeth. Zebrafish have similar genetic structure to human, 70% of human genes are found in zebra fish [16]. Any type of disease that causes changes in the zebra fish body parts in humans could be modeled in zebra fish. Zebrafish embryos are clear, which allows scientist to observe the fertilized eggs growing into a fully formed baby fish under a microscope. Researchers can easily identify and test new drugs to treat the diseases being modeled. The capability of zebra fish to generate many embryos everytime they breed makes them especially useful for drug screening [16,17,18].

Zebrafish as a model for diabetes

A disease in which the body`s ability to produce or respond to the hormone insulin is impaired resulting in abnormal metabolism of carbohydrates and elevated levels of the glucose in the blood is called diabetes [19]. Generally, diabetes is of various type-type I diabetes, type II diabetes and gestational diabetes. Type I diabetes formerly called juvenile or childhood-onset diabetes is an autoimmune condition where the immune cells of the body attack the own body cells i.e. the cells of the pancreas. This brings insulin-deficient condition in the body which requires insulin to be administered externally to maintain the blood sugar level [20]. Type II diabetes also called non-insulin dependent diabetes or diabetes mellitus previously also called adult-onset diabetes is the common type of diabetes globally. It is a condition where in the body is unable to produce insulin efficiently. It is due to poor lifestyle such as lack of physical activity or imbalanced diet which. Gestational diabetes is the type of diabetes in which there is an increased level of blood sugar/glucose but is below the diagnostic level of diabetes and is mostly observed during pregnancy. The signs and symptoms of this type are unapparent [21].

Zebra fish is capable to regenerate its damaged pancreas and restored euglycemic state similar to what would be expected in post-transplant human patients. Zebra fish possess many similarities with human beings in terms of lipid absorption, processing and metabolites [22]. The model is induced when either alloxan or streptozotocin is administered to destroy the beta cells resulting in hyperglycemia. An acute hyperglycemia zebra fish model can be induced with only water glucose or streptozotozcin. The diabetic zebra fish not only display the known human secondary complications, but they also exhibit impaired limed regeneration (caudal fin regeneration) as a consequence of hyperglycemic environment. The hyperglycemic zebra fish revert back to glycemia within 2 weeks of the drugs removal due to regeneration of endogenous pancreatic beta cells resulting in a physiologically normal glycemic state. The zebra fish provide a system to study the mitotically stable epigenetic components that support the metabolic memory phenomenon in the absence of the background noise of the previous hyperglycemic environment [23,24,25,26.] The experiments can be performed rapidly as it takes approximately 80days from diabetes induction until metabolic memory examination. Caudal fin regeneration is experimentally very approachable and allows for easy genetic manipulation for which there are vast array of tools [25,26,27,28].

Zebrafish as a model for kidney diseases and disorders

There is a close functional similarity between zebra fish and mammalian kidneys which makes zebra fish an ideal model for renal study. Zebra fishes perform glomerular filtration and tubular secretion, similar to mammalian kidney [29]. There is high similarity of functional domains between human and zebra fish

proteins. Like humans, zebra fish have a developed brush border membrane and also express megalin and cubilin receptors located in the proximal convoluted tubule segmented to aid reabsorption of salts, sugar and proteins. Nowadays zebra fish are in high demand for experimental work as they are genetically tractable and have a basic renal anatomy comparable to mammalian kidneys with glomerular filtration and tubular filtration processing. Zebra fish have the ability to develop new nephrons (nephrogenesis) in response to injury in their life span. The importance of kidney is for water excretion and the presence of odema in a mutant fish can be an indicator of impaired kidney function [30,31]. The duration of development of zebra kidney is 48 hours of post fertilization events. Fluorescent assays have been useful in studying zebra fish models of kidney function and disease. Fluorescent tracers are injected in zebra fish embryos so that they can easily be tracked under a fluorescent microscope. Tracers are also be used to asses tubular function, specially reuptake in the proximal tubule [32,33,34]. Nephrotic syndrome has also been studied in zebrafish. Zebrafish have also been used to study the effect on nephrotoxic agents sush as cisplatin and gentamicin. Injection of these drugs induces oedema in embryos as well as histological changes such as brush border flatting and debries in the tubular lumen. Injection of tracers such as dextrin and inulin into these drugs treated zebra fish showed disturbances in the glomelular filtration rate as clearance is decreased relative to the control. The hormone prolactin, which is released systematically from the pituitary gland in fish plays major role in osmoregulation. Zebra fish have been used to study the possibility of regeneration after loss of function, that nephron progenitor cells can provide new nephrons in adult zebra fish [35,36,37,38,39].The nephron progenitors persist in the adult kidney and continue to add new nephrons as the fish grows, presumably to ensure that kidney function keeps pace with increases in body mass. Therefore, zebra fish as a model for studying renal functional diseases and herbal medicine study [37,38,39,40].

Zebra fish model of human liver development and disease:

Zebrafish is used as an important model to study liver development and diseases in the past two decades. The zebra fish liver arises from the anterior foregut endoderm and therefore proper maintenance and specification of endoderm is required for normal liver development similar to mammals [41,42,43,44,45].The development and function of zebra fish organs are strikingly similar to those of humans, as they have been successfully used to model hematopoietic disorders, cancers, cardiovascular diseases, muscle disease, neurological diseases and liver diseases etc. The functional unit of mammalian liver is the liver lobule. Liver development in zebra fish and mammals can be divided into three process i.e. specification, differentiation and growth [43,44,45,46,47,48]. The liver has a remarkable regenerative capacity compare to most other organs; however, the capacity is finite and often inadequate to compensate for serve acute or chronic injury, both of which can progress to organ failure. Genes are highly conserved between humans and zebra fish, making them a useful system to study the basic mechanisms of liver diseases and perform genetic screening to identify pathways and compounds that act on specific processes. Zebra fish embryos develop rapidly, all of their digestive organs and mature in larvae by 5 days of age. At this stage, they can develop hepatobiliary diseases caused by development defects or toxin or ethanol induced injury and manifest premalignant changes within weeks [43,44,46,48,]. The liver plays a central role in nutrient and intermediary metabolism and is the major site of detoxification for xenobiotics, toxins, drugs and other exogenous substances. The zebra fish liver cells performed similar functions as their mammalian counterparts, histological stains that are routine in mammalian liver research can be easily applied to zebra fish to examine liver pathophysiology [43,44,46]. The major advantages of zebra fish as a model system for hepatic process is the ability to perform screening (genetic or chemical) in a vertebrate organism. Zebra fish have been validated as a model for *in vivo* drug discovery and methods for automated high-throughput chemical library screening continued to evolve. One important concerned in modeling human liver development and disease in zebra fish is the degree to which these models faithfully recapitulate the human condition to demonstrate the technical advantages of using zebra fish to study liver biology and discuss the necessary to gain deeper knowledge of zebra fish liver homeostasis and physiology in order to define which aspects of different liver diseases can be model in zebra fish [41,42,43,45,45,46,48].

Zebrafish: Experimental model for drug screening

Zebrafish is an excellent model organism in biomedical research, drug development and clinical therapy. Zebra fish embryos are permeable to drugs and can easily be manipulated using well established genetic and molecular approaches [49]. Overall, zebra fish's characteristic makes them ideal for fast,

reliable and low-cost drug screening during pre-regulatory phase of drug development or repositioning and they can now be used in high throughput screening (HTS) of drug libraries [50,51]. It is well suited for studies in genetics, embryology, development and cell biology and drug administration, short reproductive cycle, transparency that permits visual assessment of developing cells and organs. Zebra fish bioassays are cheaper and faster than mouse assays and are suitable for large drug screening [49,50]. There are four methodological steps for undertaking a chemical library screening in zebra fish [50,53,54,56]:

A) Adult pair mating and embryo collection.
B) Embryo sorting or arraying into multi well plates.
C) Chemical library administration.
D) Data acquisition and analysis.

The components of modern drug discovery are screening of lead compounds, target validation and assay development. Zebra fish and mammals exhibit high degree of similarity as it relates to molecular mechanisms of development and cellular physiology. Approximately 70% of genes associated with diseases in humans have functional homologus genes in the zebra fish. During organogenesis, zebrafish embryos are permeable to small molecules and drugs, providing easy access for drug administration and vital dye staining. The zebra fish genome possesses a high degree of similarity to that of humans [57,58]. The first human disease model in zebra fish was the Sauternes (sau) mutant responsible for a blood disorder involving a specific defect in heamoglobin production. Drugs effects on growth and development have been assessed by visual examination of the length and shape of the zebra fish`s body the size and morphology of internal organs, including the brain, liver, cardiovascular system, cartilage, notochord, pancreas, intestine and kidney. The use of zebra in the drug screening approach potentially facilitates fast and cost-effective identification of potential lead components and initial validation as potential therapeutics [57,58,59,60,61].

CONCLUSION:

Zebrafish as an experimental model for studying herbal medicine is very effective due to the unique characteristics of embryos, maintenance and drugs administration, short reproductive cycle, transparency that permits visual assessment of developing cells and organs. The small size of zebra fish embryos and their ability to be calculated during the first week of life make this system ideal for drug discovery and safety testing compare to rodents. The potential shortcoming is counterbalanced by several advantages relating to low cost and suitability for automated drug and toxicity testing. Zebra fish provides comparative anatomy and physiology, genome to that of humans which can be used to study initial genetic or drug target information before scaling up to expensive system moreover the transparent, larval zebra fish models can be used to study human diseases and enables rapid physiology relevant in vivo screening. The antibacterial, antiparasitic and anaesthetic drugs, besides the pharmacokinetic and pharmacodynamic parameters have been well experimented on the fish. Therefore, zebra fish used as a new model organism for different experimental studies of herbal medicines, pharmacology and toxicology.

REFERENCES

1. Chitramuthu BP. Modelling Human Disease and Development in Zebrafish.Human Genet Embryol.2013;3:e108.
2. Goldsmith JR and Christian J.Think Smail:Zebrafish as a model system of Human Pathology.J Biomed Biotechnol.2012.
3. Kishi S,et al.Zebrafish as a genetic model in biological and behavioral gerontology :where development meets aging in vertebrates-a mini review.Gerontology.2009;55:430-441.
4. Schartl M. Beyond the Zebrafish: diverse fish species for modeling human diseases. Dis Model Mech.2014;181-192.
5. Chiranjib Chakraborty,et al.Zebrafish: A complete animal model to enumerate the nanoparticle toxicity.J Nanobiotecnol.2016;14:65
6. Mathias JR. et al. Advances in Zebrafish chemical screening technologies. Futher Med Chem. 2012; 4:1811-1822.
7. Boyd RT. Using zebrafish for screening and development of new nicotinic and dopaminergic drugs.Mol Biol.2012;1:e 102.
8. Bhusnure OG,et al.Zebrafish as a model system for drug target screening and validation. J Develop Drugs.2015;4:4.
9. Spence R, G, Lawrence C, Smith C. The behavior and ecology of the zebrafish, *Danio rerio.*Biol Rev Philos Soc. 2008;13-34.
10. Major RJ, Poss KD.Zebrafish heart regeneration as a model for cardiac tissue repair.Drug DiscovToday Dis Modals.2007;4;219-25.
11. Callaway E. Zebrafish genome helps in hunt for treatments.Nature. 2013Aprl17doi 10 1038/nature.2013.12821. (Consulted May 2013) Available at http://www.nature.com/news/zebrafish-genome-helps-in-hunt-for-treatments-1.12821.
12. Bunton TE. Experimental chemical carcinogenesis in fish.Toxicol Pathol 1996;24(5):603-618.

13. Carneiro Mc.et al.Telomeres in ageing and diseases: lessons from zebrafish. Dis Model *Mech*. 2016; 9:737-48.
14. "In memory of George Streisinger, "Founding Father"of Zebrafish Development and Genetic Research" University of Oregon. Retrieved September 23,2015.
15. Langenheinrich U (2003). Zebrafish: a new model on the pharmaceutical Catwalk.Bioessays,25:904-912.
16. Lindsay MA (2003) Target discovery. Nature Reviews Drug Discovery,2(10):831-838.
17. Spitsbergen JM and Kent ML (2003): The state of the art of the zebrafresh model for toxicology and toxicologic pathology research advantages and current limitations. Toxicologic pathology 31:62-87.
18. Gerhard G.S. Kauffman E J,WangX, Stewart R , moore JL, Kasales C J, Demidenko E Cheng KC(2002). "Life spans and senescent Phenotypes in two strains of zebra fish (*Danio rerio)*" Experimental Genontology 37(8-9)1055-68.
19. A zebrafish Model of Diabetes and metabolic memory Robert V Intine AnsgarS Olsen. Michael P. sarras Dr. willam M School of Podiatric Medicine,Rosalin Franklin University of Medecine and Science
20. Ceriello, A Ihnat, MA&Thorpe,JE.Clinical review2:The "metabolic memory" is more than just tight glucose control necessary to prevent diabetic complications?*J.Clin Endocrinol.*Metab.94,410-415(2009).
21. The effect of intensive treatment of diabetes on the development and progreesion of long-term complications in insulin –dependent diabetes millutes. The Diabetes control and complications Trial Research Group.*N Engl.J med.329,977p986(1993).*
22. Turner,R.C.Cull,C.A. Frighi,V,&Holman,R.R.Glycemic control with diet, sulfonylurea,metformin,or insulin in patients with type 2 diabetes mellitus:progressive requirement for multiple therapies(UKPDS49)Uk Prospective Diabetes Study(UKPDS) Group.*JAMA*281 2005-2012(1999).
23. Gaede,P.H.Jepsen,P.V.,Larsen,J.N.,Jensen,G.V.,Parvi ng,H.H.,&Pedersen,O.B(The Steno-2 study.Intensive multifactiorial intervention reduces the occurrence of cardiovascular disease in patients with type 2 diabetes).*Ugeskr.Laeger.165,2658-2661(*2003).
24. Nathan. D.M. Cleary.P. A.et al. Intensive diabetes treatment and cardiovascular disease in patients with type 1 diabetes.N.Engi.J. Med. 353,2643-2653(2005).
25. Retinopathy and nephropathy in patients with type1 diabetes four years after a trial of intensive therapy.The Diabetes control and complications Trial/Epidemology of Diabetes Interventions and complications Research Group.N.Engl.J.Med.324,381-389(2000).
26. Amsterdam A,Hopkins N (2006).Mutagenesis in Zebrafish for identifying genes involved in development and disease. Trends Gent.22(9):437-478.
27. Bassett D, Currie PD (2004). Identification of zebrafish model of muscular dystrophy. Clin. Exp.Pharmasiol. 31:537-540.
28. Eunji Shin, Bin Na Hong and Tong Ho Kang "An optimal establishment of an acute hyperglycemia zebrafish model" African Journal of Pharmacy and Pharmacology Vol.6(42). pp2922-29218;15Nov 2012.
29. Wingert RA, Selleck R,YuJ, Song HD,Chen Z,Song A,Zhou Y,Thisse B,Thisse C, Mc Mathon AP, Davidson A J (2007) The cdx genes and retinoic acid control the positioning and segmentation of the zebrafish pronephros. PloS Genet #: 1922-1938.
30. Anzenberger U, Bit-Avragini N,Rohr s,Rudolph F, Dehmel B, Willnow TE, Abdelilah-Seyfried S (2006)Elucidation of megalin/LRP2-Dependent endocytic transport processes in the larval zebrafish pronephros.J Cell Sci 119:2137.
31. Kidney Research UK (2001) Stages of kidneys diseases.https;//www. Kidneys researchuk.org/health-information/ stages-of-kidney-disease
32. Diep CQ, Ma D, Deo,RC, Holm Tm< Naylor RW,AroraN,Wingert RA,Djordjevic G,Lichman B, Zhu H,Ikenaga T, Ono F,Englert C, Cowan CA, Hukriede NA, Handin RI,Davidson AJ(2011) Identification of nephron progenitors capable of kidney regeneration in zebrafish. Nature470:95-100
33. Christou-Savina S, Beales PL, Osborn DPS (2015) Evaluatrion of Zebrafish kidney functioning using a flurorescent clearance assay.JVis Exp 96:52540
34. Hentschel DM, Mengel M,Boehme L, Liebsch F,Albertin C, Bonvertre JV, Haller H, Schiffer M (2007) Rapid Screening of glomerular slit diaphragm intregity in larval zebrafish.Am J Physiol;Renal Physiol 293:f1746-F1750
35. 35.Tobin JL, Beales PL (2008) Restoration of renal function in zebra models of ciliopathies. Pediart Nephrol 23:2095-2099. 70 Elmonem MA, Khalil R, Khodaparast L, Arcolino FO, Baelde HJ, Van den Heuvel LP, Levtchenko E (2017) Cystinosis (ctns) zebrafish mutant vshows pronephric glomerular and tubular dysfunction. Sci Rep 7:42583
36. 36.Oltrabella F,Pietka G, Ramirez IB-R, Mironov A, Starbog T,Drummond IA, Hinchliffe KA, Lowe M(2015)The Lowe Syndrome protein OCR1 is required for endocytosis in zebrafish pronephric tubule.PloSGenet 11:1005058
37. Hentschel DM,Park KM,Cilenti L, Zervos AS, Drummond I, Bonventre JV (2005) Acute renal failure in zebrafish: a novel system to study a complex disease. Am J Physiol-Renal Physiol288:F923-F929
38. Sakamoto T, Mc Cormick SD: Prolactin and growth hormone in fish osmoregulation. Gen Comp Endocrinol 2006; 147:24-23.
39. Diep CQ, Ma D , Deo RC , Holm TM,Naylor RW,Arora N, Wingert RA, Bollig F, Djordjevic G, Lichman B, Zhu H, Ikenaga T, Ono F,Englert C, Cowan CA, Hukriede NA, Handin RI, Davidson AJ(2011) Identification of

adult nephron progenitors capable of kidney regeneration in zebrafish. Nature :95-100
40. Zhang C.Ellis JL,Yin C(2016)Inhibition of vascular endothelial growth factor signaling facilities liver repair from acute ethanol-induced injury in zebrafish. Dis model Mech9(11): 1396.doi:10.1242?dmm.024950
41. Reiter Jf, Kikuchi Y,Stainier DY. Multiple roles for Gata 5 in zebrafish endoderm formation .*Development* 128:125-135,2001
42. Tiso N, Filippi A, Pauls S, Bortolussi M, Argenton F.BMP signaling regulates anteroposterior endoderm patterning in zebra fish.*Mech dev* 118:29-37,2002.
43. Santoriello C, Zon LI (2012) Hooked! Modelling human disease in zebrafish, J Clin Invest 122(7):2337-2343.dol10.1172/JC60434
44. Si- Tayeb K,Lemaigre FP, Duncan SA(2010)Organogenesis and development of the liver. Dev cell 18(2):175-189 doi10.1016/j.devcel.2010.01.011
45. Benjamin J. Wilkins and Michael Pack, Zebra Fish Models of Human Liver Development and diseases.
46. Wolfram Goessling, Kirsten C. Sadler Zebrafish: An Important Tool for Liver Diseases Research.
47. Lu JW.Ho YJ. Yang YJ.,Liao HA, Ciou SC Lin LT et al 2015 Zebrafish as a disease model for studying human hepato cellular carcinoma. World J Gastroenterol 21(42):2042 1205 doi; 10 3748/wjg.v2114.12042.
48. Due- Hung Pham * Changwen Zhang* Chunyue Yin Using Zebrafish to Model Liver Diseases- Where Do We Stand?
49. The Importance Of Zebrafish in Biomedical Research A Importance do Preixe-Zebra na Investigaco Biomedica Barbara TAVARES1 Susana SANTOS LOPES1 Acta Med Prot2013 sep –oct;26(5):583-592
50. G kari, U Rodeck and AP Dicker Zebrafish: An Emerging Model System for Human Disease and Drug Discovery.
51. Ali S, Champagne DL, Spaink HP, Richardson MK Zebra Embryos and larve : a new generation of disease models and Drug screens.Birth Defects Res C Embryo Today. 2011; 93:115-
52. Lee H, Inselman AL Kanungo J, Hansen DK. Alternative Models in developmental toxicology. Syst Biol Reproductive Med.2012:58:10-22
53. Lessman CA. The developing zebrafish (*Danio rerio)*:a Vertebrate model for high –throughput screening of chemical Libraries. Birth Defects Res C Embryo today.2011;93:268-80
54. Chunenlei Pramg Wen Lin Semino Patricia Mc Granth Zebrafish: A preclinical model for Drug Screening
55. V.N. Sarvaiya,*K.A.Sardaria, Rana and A.M. Thaker Zebra as model Organism For drug discovery and toxicity: a review.
56. G Kari1, U Rodeck2 and Dicker Zebrafish: An Emerging Model System for Human Disease and Drug Discovery
57. Detrich, H.W.III, Westwrfield, M.&Zon,L.I. Overview of the zebrafish system. *Methods Cell Biol.59,*3-10(1999).
58. Babykumari P Chitramuthu* Modelling Human Disease and Development in Zebra fish
59. Santoriello C,Zon LI(2012) Hooked! Modelling human disease in Zebrafish, J Clin Invest:122:2337-2343.
60. Lieschke GJ,CorriePD(2007)Animal models Of human disease: zebrafish swim in view .Nat REV Genet 8: 353-367.
61. Gates MA, Kim L,Egan ES Cardozo T, Sirotkin Hl ,et al .(1996) A Genetic linkage map for zebrafish: comparative analysis and localizatiob of genes and expressed sequences. Genome Res 9:334-347.