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Zebrafish an emerging model for Preclinical Drug Discovery

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Abstract

For understanding the pathogenesis of human disease at cellular and molecular level bio medical research depends on the use of animal models. Maximum animal models used in medical research of human disease are basically performed in murine system. Though there are advantages of using these animals, murine's have long gestational period, sexual maturation rate and are expensive. These invertebrates lack many structures and organ systems that are involved in human disease pathogenesis. Forward-genetic screens and random mutagenesis based reverse genetics though possible in mouse and are currently underway; they cannot be done on a large scale as they require considerable staff and infrastructure support. Due to these drawbacks other animal models have been developed that provide initial genetic or drug target information. For developmental genetic studies larval zebrafish are used extensively due to their small size, external development, optical transparency, and accessibility in large numbers. Not only are they vertebrate, they also have high fecundity, which can be easily visualised and experimentally manipulated. 70% of all human disease genes have functional homologous in zebrafish. There has been significant increase in the use of zebra fish to elucidate the etiology of human disease like cancer, infectious diseases, cardiovascular disease, kidney disease, diabetes, blindness, deafness, digestive diseases, hematopoiesis, muscle disorders and neural disorders.

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INTRODUCTION

Maximum animal models used in medical research of human disease are basically performed in murine system. A lengthy gestation period (18–20 days) and rate of sexual maturation (6–8 weeks) when mutualised with high cost of housing and breeding results in significant boundaries in spite of the knowledge produced by these murine models (Lieschke and Currie, 2007). Experiments with mice are laborious. Hence, they are not suitable for high-throughpout screening. These limitations have led to the development of other animal models that provide initial genetic or drug target information and will not be expensive (Goldsmith and Jobin, 2012). For studying the developmental biology zebrafish has been considered as an ideal model. Thus, it is a vertebrate akin possessing much similarity to humans compared to any model which consider the invertebrates, with much benefits over other models including vertebrates. As it possesses better rate of reproducibility (a few hundred eggs per spawning) with transparent embryos, and offers visualization of external development, the experimental models can be easily manipulated. The generation time being short (2–3 months) all of these features, previously found mainly in invertebrate models, simplify

the genetic and high-throughput functional studies (Kawakami, 2005; Xi *et al.*, 2011; Tavares and Lopes, 2013). River Ganges is the main origin source of Zebrafish. In Latin, the zebrafish is known as Danio rerio and is very common in aquariums around the globe (Lawrence, 2007). The genetic map of zebrafish demonstrates highly conserved similarities with the human genome. Roughly 70% of of the complete human genome has its functional homologs in zebrafish (Santoriello and Zon, 2012). The development of a zebrafish is rapid and most of the organ systems are completely developed 5 days post-fertilization (dpf). Due to the rapid development and especially the smaller small size, the zebrafish is particularly suitable for the testing of chemical compounds that may challenge the developmental pathways (Peterson *et al.*, 2001) and disease mechanisms (Peterson *et al.*, 2004). Zebrafish has become one of the important research tools and is also used for elucidating the etiology of human disease. Zebrafish is the ideal model for the study of various ailments in humans like cancer, infectious diseases, cardiovascular disease, kidney disease, diabetes, blindness, deafness, digestive diseases, hematopoiesis, muscular disorders and neural disorders. Few examples have been highlighted below for the better understanding of why zebrafish is being used to develop a model, explore disease biology and to develop newer principles of therapy (Kar, 2013).

ZEBRAFISH AND DISEASE MODELS

Wound Healing/Restitution

Wound healing is characterised by critical biological response of injured tissues and organs (Richardson *et al.*, 2013). One of the most popular zebra fish wound model is larval tail wounding model, where a section of tail fin is resected.

A complete regeneration of the heart is possible in zebrafish, after a 20% resection of the organ, within 2 months (Goldsmith and Jobin, 2012) and hence are used for their ability to regenerate cardiac tissue. Zebrafish larvae expressing a genetically encoded H_2O_2 sensor showed that H_2O_2 signals to leukocytes in tissues, in addition to its known antiseptic role indicating the role of H_2O_2 during the early events of wound responses in Zebrafish (Niethammer *et al.*, 2009).

Gastrointestinal Diseases

The gastrointestinal system of a zebrafish is homologous to humans and consists of pancreas, liver, gall bladder. The secretory and absorptive functions are carried out by the intestinal tract similar to humans. This has led to the development of various pathological models of the gastrointestinal tract in the zebrafish (Annie *et al.*, 2005; Wallace *et al.*, 2005). For the assessment of prokinetic drugs and GI motility, the zebrafish larva motility model has been very useful (Zhou *et al.*, 2014). Owing to the similarities in the physiology and pathological conditions of the gut, various human diseases can be easily modelled into the zebrafish. To study the pre mammalian gastrointestinal diseases by high throughput screening and genetic manipulation zebrafish is the ideal model (Fleming *et al.*, 2010).

Cancer

Histologically there is high degree of similarity between zebrafish and human tumour. To develop cancer models in zebrafish the employment of chemical treatment, transplantation of mammalian cells, forward genetic screens, and reverse genetic approaches using knockouts and transgenes have been widely accepted (Santoriello and Zon, 2012). Approximately 1:100 high locus mutation rate is produced by gamma ray mutagenesis and hence has been used in screening morphological defects (Haffter *et al.*, 1996). The zebrafish is known to exhibit large deletions and translocation in the genome on introduction to high frequency gamma rays. Thus, chemical mutagenesis is the first-choice method to induce mutagenesis. The process of insertional mutagenesis involves injection of a plasmid DNA, a mouse pseudo typed retro-virus or using a P-element transposon . These genetic screening helps in elucidation of novel genes and mutants for specific organs or processes (Driever *et al.*, 1996).

Cell survival and apoptosis

Major part of the normal development of many organ systems and tissues involves apoptosis. The usefulness of zebrafish in studying early development, and the ability to label apoptotic cells due to recent advancement in technology has made it possible to visualize apoptotic cells in this model system (Cole and Ross, 2001). Apoptosis is known to be modulated in vivo by a plethora of smaller molecules and here lies the current interest of pharmacology. Several investigators studied apoptosis mechanisms in zebrafish embryos due to the high degree of evolutionary conservation of molecular processes governing regulation of apoptosis (Eykelbosh and Kraak, 2010). For example, zebrafish DNA fragmentation in vitro was inhibited by peptide-based caspase inhibitors of both invertebrate and vertebrate homologues of caspases 1, 4, and 5 or caspases 2, 3, and 7. It has also been seen that in early zebrafish development caspase inhibitors

prevent nocodazole-induced apoptosis. These data suggest that caspases of the zebrafish share similar substrate-recognition sites with their mammalian homologues and perhaps target the same proteins in vivo. During the development of a zebrafish embryo detection has been done for apoptotic events specific to the organs and tissues. For the identification and detection of apoptosis in the embryos using high-throughput screening, acridine orange dye is extensively used (Kari *et al.*, 2007).

Angiogenesis and vasculature

An excellent target for cancer therapy is inhibition of angiogenesis since formation of vessels is necessary for tumor growth. Any small molecule affecting the process of angiogenesis can be screened viably by using zebrafish models. Prominent patterning of blood vessel can be visualized microscopically useful in screening for compounds that affect angiogenesis by staining the sub-intestinal vessels (SIVs) (Serbedzija *et al.*, 1999). Vascular system development has been well established in zebrafish embryos (Rubinstein, 2003). Major vessels formation is complete along with beating of heart, ongoing circulation, and angiogenesis in the somites and the anterior portion of the embryos. It is by angiogenic sprouting that blood vessels are formed in many zebrafish and they also seem to require the same proteins that are essential for the growth of blood vessels in mammals. Using endothelial cell-specific promoters such as KDR/Flt, transgenic zebrafish have been developed in which green fluorescent protein (GFP) expression is directed to endothelial cells. There is no immediate lethal effect due to defective vessel formation because embryos can survive and develop for up to a week without an intact circulatory system. This permits thorough analysis of the effects of pro angiogenic compounds (Kari *et al.*, 2007).

Cardiac Disease

In vivo models provide a very low throughput in exchange for a considerable expense, whereas, the current in vitro assays performed preclinically are biologically simple. On testing of 100 small molecules for their effects on chronotropicity in the heart of zebrafish, it was found that the drugs known for causing QT prolongation in humans consistently caused bradycardia and AV block in the zebrafish. Heritable modifiers of such drug effects can be explored in zebrafish due to their genetic tractability (Milan *et al.*, 2003).

For study of various heart models such as heart development, heart muscle growth, endocardium and valves development, cardiac conduction system, cardiac electro-physiology, heart regeneration zebra fish models haves been used (Poon and Brand, 2013) . They are used for study of a wide variety of cardiovascular disease processes which includes congenital heart defects, arrhythmia, cardiomyopathy, aortic coarctation. The characteristic optical clarity of zebrafish enables it to be used for the studies on valvulogenesis and detailed electrophysiological mapping to screen the conduction system of the heart. Zebra fish larvae oxygenate through diffusion only which is a very unique character. This allows the study of mutations causing severe cardio myopathy phenotypes such as *silent heart* and *pickwick*^{m171}, which mimics titin mutations observed in human dilated cardiomyopathy. The capability of the cardiac tissue in the subject to regenerate allows the formulation of new therapeutic strategies for cardiac injury, including scar formation following myocardial infarction (Bakkers, 2011; Asnani and Peterson, 2014).

Myocardial infarction

Irreversible loss of heart tissue mainly characterized in, myocardial infarction, theand the tissue is replaced with a fibrous scar. In contrast to the partial resection model, cryoinjury heart infarct model causes serious cellular mortality similar to that of the mammalian infarcts mainly observed in 20% of the ventricular heart wall (Chablais *et al.*, 2011).

Cre/lox lineage tracing system have been used to locate the source of the newly formed cardiomyocytes following this injury. It was found that cardiomyocytes which experience partial dedifferentiation gives rise to the regenerated cardiomyocytes characterized by structural changes and expression of cell-cycle progression genes. According to the study, these data suggest that zebrafish heart regeneration is driven by pre-existing cardiomyocytes rather than by progenitors, as previously suggested. A small ubiquitous protein, Thymosin b4 is presumed to be a signal which is known to trigger the formation of new cardiac tissue and blood vessels in fish. These observations enlighten the path towards finding new drugs for therapy of heart injury in zebrafish (Jopling *et al.*, 2010).

Diabetes

In diabetes research the adult zebrafish has become the potential and important model. Genes responsible for the regulation of blood glucose in zebrafish has been identified and it has also been found that the mechanism of blood glucose regulation in zebrafish by insulin release is very much similar to that in mammals. Similar to mammalian model adult zebrafish respond to anti-diabetic drugs, by reducing blood glucose levels (Elo *et al.*, 2007; Eames *et al.*, 2010). The working of insulin promot-

ers and insights into diabetes for rodent genes have been investigated. The reports illustrate significant dissimilarities between rodents and human promoters and their genes. The similarity of insulin genes in zebrafish and humans authenticates the key importance of the insulin hormone product (Olsen *et al.*, 2012).

It's a complex and stepwise process of development of the vertebrate pancreas. In zebrafish differentiation of the pancreatic cells are mediated by extrinsic signaling molecules which influence the intrinsic transcriptional programs. Experiments on zebrafish has revealed several signaling molecules responsible for this differentiation process (Kinkel and Prince, 2009).

In addition to hormones, nutrients like lipids and carbohydrates are also responsible for the regulation of transcription of the genes coding for glycolytic and lipogenic enzymes. They execute their functions primariely through the cellular nutrient/energy receptors. Identification of such nutrient/energy receptors is done by using fasting blood glucose level as a parameter from the mammalian studies. Zebrafish is an important model system for studying the human metabolic disorder (Craig and Moon, 2011). The mechanism for regulation of gluconeogenesis in both mammals and zebrafish are the same. Expression of phosphoenolpyruvate carboxykinase (PEPCK) regulates insulin levels and glucagon is responsible for the regulation of the rate limiting step of gluconeogenesis. Changes in PEPCK expression can be obtained through real-time PCR analysis of whole larval RNA (Elo *et al.*, 2007).

Lipid research

To study the biological phenomena, Zebrafish are an increasingly popular vertebrate model organism. To study the lipid-related diseases, model that including atherosclerosis, obesity, diabetes and hepatic steatosis Zebrafish is used as an ideal model system (Hölttä-Vuori *et al.*, 2010).

In obesity endocrine signaling and lipid homeostasis is altered by dietary and xenobiotic compounds. Examination of the effects of diet, drugs and environmental contaminants on the white adipose tissues singly or in the combined form can be done by zebrafish obesogenic (ZO) test which is a short-term assay method. Obesogenic and anti-obesogenic compounds and their mixtures provide relevant information by whole organism testing. The ZO test, which provide the information on adipocyte lipid droplet size and adiposity as its endpoints (Tingaud-Sequeira *et al.*, 2011).

Regeneration Studies in zebrafish

A perfect coordination in the proliferation and patterning of mature cells following any severe injury or amputation provide the information regarding complex tissue regeneration process. As compared to mammals certain lower vertebrates have a superior regenerative capacity. Very little information is available regarding the molecular and cellular mechanisms of regeneration. Zebrafish is a suitable model system to overcome this deficiency. The regenerative process refers to perfectly or near perfectly replacing of damaged or lost structures. In mammals, majority of organs heal by scarring. In the process of understanding the regenerative medicine for humans the first leap is the better understanding of the regenerative process in lower organisms. Zebrafish is a teleost fish that can regenerate multiple structures like fins, optic nerve, scales, heart, and spinal cord which is open to standard molecular and genetic manipulations (Poss *et al.*, 2003; Curado *et al.*, 2007). For investigating the heart regeneration a model developed by using zebrafish embryo/larva provides the greatest of advantages, during first week of development the embryo/larva oxygenates only through diffusion thus tampering with its cardiac function within that period does not risk the survival of the subject (Stainier, 2001). Regeneration studies in multiple tissues of zebrafish have helped identify new mechanisms and has served as a guide for anticipating regenerative strategies in mammals (Gemberling *et al.*, 2013).

Neurodegeneration

Central nervous system disorders in humans include Parkinson's disease, schizophrenia, Alzheimer's disease, and depression. Aminergic neurotransmitters are found to be readily involved in the pathogenesis of these disorders. On comparison it was found that the central aminergic system of humans and zebrafish are considerably similar. There is highly similar noradrenergic, serotonergic, and histaminergic system. Similar dopaminergic systems come to exist. A major difference is observed in the mesencephalon of zebrafish where it is lacking dopaminergic neurons. . By alterations of brain dopaminergic systems with MPTP, not only alterations in the dopaminergic system is observed but it also shows abnormal motor behavior. An alteration in the histaminergic neuron networks along with effect on memory and swimming was observed when histamine deficiency was induced chemically. With the help of imaging techniques and behavioral methods zebrafish genetics study reveal how the important behaviors are produced by interaction of modulatory transmitter systems, and in what way they are regulated in disease conditions and modulate pathophysiology (Panula *et al.*, 2006; Bandmann and Bur-

ton, 2010). Detection of alterations in basic motor function, changes associated with exteroceptive and interoceptive sensory cues, and alterations in learning and memory performance by using various test methods available, helps in study the altered neurological function. Zebrafish is the ideal model system for study the many of the behavioral disease models which are similar in mammals (Tierney, 2011). Zebrafish provides the most considerable vision into the formation of sensory circuits, and exhibit great potential for explaining the functioning of sensory systems at an organismal level (Guo, 2004).

Proconvulsant activity of PTZ and the combination of PTZ with proconvulsive caffeine was estimated by the latency of seizure produced in zebrafish (Gupta *et al.*, 2014). Locomotor response of nicotine was studied in larval zebrafish (Cousin *et al.*, 2014).

Drug Development process (or discovery)

Testing of drugs in a large scale in mammals requires time and capital thus making the process not so reasonable. Such a method can only be appropriate for application into lower group animals or merely cultured cells. However, when such tests or assays are carried out in lower organisms or cells it portrays a major disadvantage. Such organisms often are not capable of portraying a human disease and even if the disease is induced, the measure of outcome likely to provide relevance for the identification of drugs to be applied into the clinical setting is not enough. The smaller size, rapid rate of development, ease of availability of the zebrafish brands them advantageous in the drug discovery processes. Zebrafish screens in melanoma achieved the greatest success, wherein as the result of tremendous research a novel drug has made it as far as upto the clinical setting and has stimulated the sanguinity that similar screens of other diseases will harvest parallel results (White *et al.*, 2011). To study the toxicity much earlier in drug development process, high-throughput zebrafish toxicity assays model was established (Parng, 2005; Zon and Peterson, 2005). The most pertinent distinguishing property of zebrafish embryo assays and also their integrative capacity, it allows the resolve the wide range of effects in a single system. Image-based high-throughput methodologies, due to its developmental characteristics and transparency allow to screening of the large number of compounds in preclinical research purpose (Raldúa and Piña, 2014). These screenings can be performed with reasonable effortlessness, as chemicals can be simply added to the water. A high throughput screening for small molecules from the available libraries a plethora of compounds have been identified which can potentially make it to the clinical setting due to their suppressing effect on the altered phenotypes (Bassett and Currie, 2004; Zon and Peterson, 2005). Various new compounds are currently under clinical trials which have been screened in the past few years (Wiley *et al.*, 2017).

Immuno and Inflamation

There is a high similarity between the human immune system and zebrafish immune system (Traver *et al.*, 2003). Complete development of the immune system (innate or adaptive) is followed even in the small zebrafish. The growth and development of immune system was easily observable due to its transparent embryos. using whole mount in situ (WISH) in fixed embryos, or followed in real time in live transgenic fish in which fluorochromes have been used to tag or tracked cells. The inflammatory response which follows an injury directs the immune cells of the host system to tackle pathogens and sustain integrity of the tissue in consideration. Variety of signal gradients established the immune cell recruitment to the site of inflammation (Redd *et al.*, 2006). After injury in zebrafish embryo demonstrated that the wounded cells instantaneously start to produce hydrogen peroxide (H_2O_2) which is none other than a signaling molecule for the leukocytes (Pase *et al.*, 2012). Hematopoiesis studies suggest that maximum cells possess the qualities equivalent to that of the zebrafish but exceptions exists (Stachura and Traver, 2011). In both mammals and zebrafish, the thymus is responsible for production of T lymphocytes. The only difference that prevails is in the morphology of the gland. In zebrafish the gland remains as two separate bilateral structures (Lam *et al.*, 2002). The innate immune system: the acute phase response to infection, the interaction of host and pathogen, and the chemotactic response to injury the zebrafish is the ideal model to study. Due to higher similarities of zebrafish to human and other mammals. Therefore, a surge in the immuno models of zebrafish is noted, providing new understandings in the development of the immune system and its functioning (Meeker and Trede, 2008), and also to study leukocyte recruitment and inflammation Zebrafish is an powerful model system (Lieschke and Currie, 2007; Mathias *et al.*, 2009).

Muscle disorders

Zebrafish models for human muscular disorders stands beneficial due to some specific qualities. Its reproducibility, easily measured motor behaviors are the qualities that are present from the first day of the life. Mammalian skeletal muscle is struc-

turally very similar to that of the zebrafish and shares identical histological and molecular features. Such striking similarities includes conservation of the components of the dystrophin-associated glycoprotein complex, contractile apparatus and the coupling machinery for contraction-relaxation. These structures happen to be the most vital muscular structures required to understand any muscle disease pathophysiology (Guyon *et al.*, 2003; Dou *et al.*, 2008). In a growing zebrafish, skeletal muscle is the largest organ and can be easily visualized. As a final point, the zebrafish models are capable of precisely estimating the severity of clinical condition compared to corresponding rodent models (Berger *et al.*, 2010; Berger and Currie, 2012). Mutations of SepN1, a selenoprotein, and RyR1, the major component of the ryanodine receptor regulated intracellular calcium channel, result in an overlapping spectrum of congenital myopathies. The immediate developmental and molecular roles of SepN and RyR *in vivo*, loss-of-function effects were analyzed in the zebrafish embryo. It was demonstrated that these two proteins are required for carrying out the normal calcium fluxes in the embryo and also for the same cellular differentiation events. The absence of either SepN or ryanodine receptors are known to cause similar diseases in the human muscle and the zebrafish embryo (Jurynec *et al.*, 2008). Mutagenic screens of the zebrafish genome have led to the identification of a class of recessive lethal mutations in which muscle differentiation occurs normally, which is charted by tissue-specific degeneration reminiscent of human muscular dystrophies. It was seen that, results from mutations within the zebrafish orthologue of the human *Duchenne muscular dystrophy* (*DMD*) gene causes *sapje* (*sap*) mutation. Mutations in this locus cause Duchenne or Becker muscular dystrophies in human patients and are thought to result in a dystrophic pathology by disrupting the link between the actin cytoskeleton and the extracellular matrix in skeletal muscle cells (Bassett and Currie, 2004; Kawahara *et al.*, 2011).

Kidney Disorder

Polycystic kidney disease (PKD) is a common genetic disorder which is characterized by formation of multiple cysts in the kidneys. These cysts are thought to be the result of excessive proliferation of epithelial cells. Similar conditions can be developed by a zebrafish leading to its significance in the research revolving around the disease (Sun, 2004).

Zebrafish embryonic kidney is structurally simple which enables it to become a popular model for the study of renal organogenesis and the search for new therapeutic strategies. Studies using zebrafish has shown significant advancement in the discovery of the nature and disease severity for Acute Kidney Injury (AKI) and ciliopathies of the kidney (Swanhart *et al.*, 2011).

Gene regulatory networks in the control of kidney development if explained would provide an insight on the origin of birth defects related to the renal system, which will further aid in the clinical interventions for such conditions. Nephrons are the basic microscopical and functional unit of kidney. Podocytes are specialized endothelial cells located on the nephrons that houses the blood filter, otherwise known as the glomerulus. Any dysfunction of the kidney is bound to arise due to damage to these podocytes. Interaction of podocytes with the vasculature produces sieve like structures that collects and filters the fluid. Filtrate enters the nephron which changes it to urine. Podocytes are also responsible for the protection of nephrons as it prevents the entry of high molecular weight proteins and larger cells. Thus, any damage to podocytes can lead to renal diseases like chronic kidney disease. In zebrafish the genetic intervention required for the development of podocyte if understood, is likely to be applicable to a wide range of vertebrates and mammals (Wingert and Kroeger, 2014).

As a response of various renal diseases there is either partial or complete loss of kidney functions. In mammals the damaged nephrons can be repaired only partially and new nephrons cannot be produced (Humphreys *et al.*, 2008). Whereas, a zebrafish can produce nephrons throughout their complete lifespan and can even regenerate them following an injury. Thus, a model if developed can provide an insight on how can mammalian regeneration of nephrons can be activated (Reimschuessel, 2001; Zhou *et al.*, 2010). These data when integrated concludes that the zebrafish kidney is likely to contain nephron stem cells. If these cells could be identified by any means then mammalian cells with similar properties could be isolated or developed which would provide a gateway to therapeutic regeneration of the nephrons in mammals (Diep *et al.*, 2011).

Homeostasis in the body is primely maintained by the renal system. Nephrons are responsible for various homeostatic functions like removing metabolic waste from the body, fluid regulation, and maintaining the electrolytes balance. AKI is liable to cause the loss of these activities and AKI can occur in response to toxins and ischemia mediated organ damage. The ability to regenerate or repair damaged nephrons following AKI in humans is not com-

pletely understood. However, researchers studying AKI in vertebrate animal models such as mammals, and more recently the zebrafish, have documented robust regeneration within the nephron blood filter and tubule following injury.

Anatomical simplicity of their kidneys has made zebrafish a popular model for studying renal diseases. Zebrafish as a model has been established for polycystic kidney disease (PKD), nephronophthisis, acute kidney injury (AKI), various other ciliopathies (Swanhart *et al.*, 2011).

Leukemia

A high degree of genetic and morphological similarity in hematopoiesis between the zebrafish and human indicates that zebrafish can provide valuable knowledge about the mechanisms behind pathogenesis of leukemia (Teittinen *et al.*, 2012).

About 60% of cases, mutated *NOTCH 1* gene found to be responsible for T-cell acute lymphoblastic leukemia (T-ALL). To study the molecular level mechanism of human NOTCH1-induced T-cell leukemia transgenic zebrafish model have been used. The purpose of this model is to detect a strong interaction between *NOTCH1* and *bcl2* suggests that genetic modifier screens have a high likelihood of revealing other genes that can cooperate with *NOTCH1* to induce T-ALL. Genetic modifier screens using transgenic zebrafish prone to NOTCH1-induced T-cell leukemia may ultimately reveal suppressors or enhancers of Notch pathway in T-ALLs. These modifier genes could be the targets for the development of new therapies (Chen *et al.*, 2007).

To study the mechanism of tumorigenesis, Zebrafish is valuable research model. The model organism is induced to develop T-cell leukemias resembling those in humans and is amenable to large-scale forward-genetic screens that can be used to uncover novel cancer pathways (Langenau, 2003).

Comparisons of zebrafish and mammalian hematopoiesis and lymphopoiesis indicate that the genetic programs underlying vertebrate blood development have been highly conserved through evolution (Thisse, 2002).

Acute lymphoblastic leukemia (ALL), a disease with relatively homogeneous morphology and immunophenotype, was another type of cancer found in zebrafish but with great heterogeneity at the genetic level, which can lead to distinct responses to therapy. One of the main causes of ALL is the TEL-AML1 fusion, associated with B-lymphocytes. Transgenic fish were generated from this mutation in all cell lineages, and developed lymphoblastic leukemia, which phenocopied the childhood CD10+, pre-B ALL. Creation of a transgenic line for the oncogene Myc led to the development of T-cell mediated ALL. Other mechanisms responsible for ALL have also been addressed with zebrafish, such as Notch1-induced T-ALL (Tavares and Lopes, 2013).

Alzheimer's Disease

One of the most significant neurodegenerative disorder is Alzheimer's disease. After extensive research our understanding of the disease still remains incomplete. Both an effective treatment and the validity of the existing disease models are still under question. The zebrafish (and, in particular, its embryos) is a malleable and accessible model possessing a vertebrate neural structure and genome. Zebrafish genes orthologous to those mutated in human familial Alzheimer's disease have been defined (Newman *et al.*, 2011). The characteristics of these genes are difficult to observe in any model but in zebrafish some characteristic discoveries have been possible. Zebrafish possess genes orthologous to all the genes known to be involved in Alzheimer's disease (Chen *et al.*, 2007).

Although zebrafish lacks the complexity of advanced cognitive behaviors evident in rodent models, they have proven to be a very informative model for the study of human diseases. Zebrafish possess genes orthologous to those mutated in familial Alzheimer's disease and research using zebrafish has revealed unique characteristics of these genes that have been difficult to observe in rodent models (Newman *et al.*, 2014).

The presence of various neurotransmitters, both excitatory and inhibitory, is reported in the central system of zebrafish. A tight junction-based blood brain barrier with macromolecular permeability is also found in Zebrafish which is useful to study about the novel AD drugs (neuroprotective drugs).

Future perspectives

Zebrafish is believed to have a strong hold in the study of diseased genes and identifying newer therapeutic strategies. This notion has been picked up due to the unique and very versatile features of zebrafish.

Interfacing with human genetics and genomics

The potential genes causing disease in humans have been identified mostly by complete genome screening and the genome association studies. Zebrafish is well known for the functional depiction of such genes and further can be used to investigate coding and noncoding function in vivo. Several studies are conducted to verify the association of genetic

variants in the pathogenesis of many diseases in the zebrafish by implying to the genetic approach (Santoriello and Zon, 2012).

Transplantation of tissues

Cell and tissue transplantation techniques have been used to investigate the mutant embryos with defective tissues. Model for tumor transplantation assay using zebrafish is quite reliable. Tumor cell reprogramming, migration, metastasis, and effects on vasculogenesis can be studied by cancer cell lines which can be the derivatives of different tissues or species (Santoriello and Zon, 2012).

Gene function involved in physiological processes is quite similar in fish and humans. Zebrafish models are a much convenient means for the investigation and identification of the genetic markers which can either suppress or enhance the disease conditions (Chakraborty *et al.*, 2009).

Zebrafish models stand unique by being transparent and possess strong potentiality to fulfil this important function in the study of human disease, enabling rapid, physiologically relevance in in vivo screening. The transparency of zebrafish also allows for screening of pathophysiology by various imaging methods which has proven to be very useful in understanding numerous disease mechanisms.

The zebrafish models stand on an underdeveloped platform with uncovered potentials. With growing understanding of the genetic mechanisms, anatomy and physiology of zebrafish the importance of this model will grow to become a powerful element of various researches involving the murine system. Extensive research on zebrafish has proved its supremacy as a model for human diseases over other conventional models. The unique features of zebrafish provides as the most appropriate in vivo system for investigating the preliminary stage of any pathology before the observations are translated to other expensive murine systems which will conserve both time and capital (Goldsmith and Jobin, 2012).

CONCLUSIONS

The present investigation mainly focuses on establishing zebrafish as an alternative preclinical drug discovery model. Screening of toxicological profile of compounds can be easily done in zebrafish due to its high visibility at developmental stages. Zebrafish also used to investigate the causes and pathologies of human diseases like cardiovascular, neurological, wound, gastrointestinal, diabetes. Because of its highly similar genome with human provided the support to zebrafish as an ideal model system for human diseases.

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