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A Review on Zebrafish Morphology and as a model for Cancer (Chemotherapy)

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Abstract: *Zebrafish (danio rerio) represent vertebrate model organism. It share a high level of genetic and physiologic homology with humans. They are small in size having length about 2 to 5 cm long. Young, middle-aged and old zebrafish, 20-29, 36-48 and 60-71% of average life span respectively. The main benefits of using zebrafish as a toxicological model over vertebrate species are with regards to their size, husbandry and morphology and finical easily availability. It was used in human cancer since 1960. Zebrafish cancer model have been developed by no of mechanism like chemical carcinogenesis, forward genetic screens, reverse genetic screens, transgenic model xenotransplantation in embryo. Xenotransplantation of human cancer cell into zebrafish embryo has also proven to be useful tool in cancer research. For all of the above-mentioned resons, zebrafish is approaching a future of being a preclinical cancer model.*

Keywords: *Danio rerio, Elosmoid scale, Retroperitoneal, Xenotransplantation, Zebrafish.*

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I. Introduction:

Danio rerio known as zebrafish, since it was first described by "Francis Hamilton" from the Ganges Delta in 1822. It is tropical freshwater fish native to the northern Indian subcontinent. Zebrafish are small in between 2 to 5 cm long. Male zebrafish are slender and torpedo shaped, with black longitudinal strips and usually a gold coloration on the belly and fins. Female can be distinguished from males because of their bigger underbelly.

Zebrafish share a high level of genetic and physiologic homology with humans, it have organ systems such as Integumentary, Skeletal, Nervous, Cardiovascular, Respiratory, Digestive, Sensory, Reproductive and Excretory System because it used for biomedical researchers in human diseases like metabolic disease, lung disease, neurological disease and genetic disease. In the metabolic disease includes nonalcoholic fatty liver, type 2 diabetes mellitus, dyslipidemia and other hepatic diseases. In the lung diseases mainly used for asthma and COPD. Neurological disease include Alzheimer's and Parkinson's diseases. Genetic diseases such as cancer.
Zebrafish is powerful model for human cancer. In 1960, zebrafish was firstly used in human cancer research. The first human Xenotransplant assay in zebrafish beginning in 2005.

PHYLOGENY OF ZEBRAFISH:

Category	Taxon
Kingdom	Animalia
Phylum	Chordata
Class	Actinopteri
Order	Cypriniformes
Family	Danioridae
Genus	Danio
Species	Danio rerio

Morphology and physiology of zebrafish (danio rerio):
External anatomy of zebrafish (dainio rerio)

The Structure of zebra fish mainly divided into four parts such as body, head, trunk, tail. Head is smaller than trunk. In the Body configuration present mouth, operculum, vent, coelomic cavity and internal organ. Sensory organ contain nostril, barbel, eyes, lateral line. Under the skin containing general colaration, scales, general pattern and type, mucous (slime layer). Fins of zebrafish divided into pectoral, dorsal, pelvic, anal and caudal fins. Zebrafish do not have a diaphragm physically separating a thorax from an abdomen the coelomic cavity holds all of the internal organ of the fish.

The organ system of zebrafish includes:
Integumentary system of zebrafish

The skin of zebrafish is traditionally divided into three layer are epidermis shows the ultimate boundry between the fish and environment. In adult skin there are two to eight layer of super basal cells over the surface of the fish. The epidermis harbors contain additional cells are goblet cells secret mucous, club cell produce alarm substance, ioncytes and chemosensory cells maintaining chemical homeostasis and somatosensory cells enabling sence of touch. The dermis contains dense collagenous stoma and the stratum compactum dermis present scale forming cell, scale pocket cell, dermal fibroblast, calcified scale plate and collagenous stoma. Development of Elosmoid scale hundreds of arrow head shaped, calcified scale are embed in the dermis of adult zebrafish. Zebrafish easily recognized by their horizontal strips which are formed by pigment pattern of three types of pigment cells are melanophore, xanthophore and ididophores. The structure of scale plate consist of a basal layer of weakly ossified external layer.

Skeletal system

The adult skeleton of zebrafish is relatively complex and once full formed by around 2 months is composed of 74 ossified cranial elements. 28-31 vertebrae, 4 cervical, 10-11 thoracic vertebrae and 15-16 separated vertebrae in the tail region and fins. The axial skeleton is derived from somatic mesoderm and the vertebral column develops from sclerotome, a mesenchyme cell. Sclerotome cells contribute to the column in the zebra fish move to surround axial midline structure. In the zebrafish axial skeleton includes the vertebral column and associated median (unpaired) fins. Vertebral column divides into precaudal and caudal vertebrae, the precaudal vertebrae are composed of centra, neural arches, spines, parapophysesand ribs, the caudal vertebrae are composed of centra, neural arches, neural spine, hemal spines and hemal archs of vertebrae.

Nervous system of zebrafish

Nervous system of zebrafish includes two major systems are central nervous system and peripheral nervous system. In the central nervous system consist spinal cord which is most caudal part of CNS, rhombencephalon (hind brain prosencephalon (fore brain) it is largest area of the brain, mesencephalon (mid brain). The peripheral nervous system divided into two class somatic and autonomic nervous system.

CNS

Rhombencephalon (hind brain), the zebrafish rhombencephalon is characterized as in all vertebrae by its association with the majority of cranial nerves and their primary motor and sensory centers. The precise organization of the vascular system of the zebrafish brain but as in the other vertebrates it is likely to represent a major energy supplier for the neurons. Cerebrospinal fluid circulate in the ventricular system, which significantly modified as compared to tetrapod. Cerebrospinal fluid produce by the choroid plexus.

Prosencephalon (fore brain) divided into different parts telencephalon are sub divided into ventral sub pallium and dorsal pallium. Hypothalamus also part of the prosencephalon, hypothalamus consist of the ventral part of secondary prosencephalon , function of prosencephalon are hypothalamus contains sensory receptors for chemical detection such as osmoregulation, ionic transport, skeletal growth, tooth regeneration, regulation of sleep, heart rate, blood pressure and the control of body temperature. Diencephalon is corresponds to the three first prosomers of the prosencephalon , they are from posterior to anterior pretectum and anterior to it the epithalamic structure, dorsal thalamus with its anterior, dorsal posterior, central posterior nuclei and ventral thalamus with the ventro lateral and ventro medial nuclei.

Mesencephalon (mid brain) comprised three main component like other vertebrae first component is cerebral peduncles contains fiber bundle from the telencephalon, second component is tomentum contains red nucleus nerve nuclei and parts of the reticular formation, last component is tectum plays major role in controlling eye movement fine motor programs and sensory motor coupling. The layering of the zebra fish tectum as for other teleost is six tangential layer. Stimulation of different region of tectum in freel moving of fishes resulted in body reorientation, turning or rolling to one side or other and some escape movement.

PNS

The peripheral nervous system of vertebrae encompasses the sensory neurons. The somatic nervous system is the part of peripheral nervous system, it providing information about muscle and limb position and receiving external stimuli. The autonomic nervous system is also part of peripheral nervous, they are classified into sympathetic and parasympathetic nervous system. The sympathetic nervous system originates from ventrally migrating neural crest cells. The parasympathetic nervous system is largely derived from mesencephalon neural crest, they controlled inflammation of swim bladder and gas secretion

Sensory organ of zebrafish (danio rerio)

Eye The mesencephalon is relatively large and automatically subdivided into the optic tectum which forms the roof of the third ventricle and the tegmentum. The optic tectum is relatively large and is divided by a longitudinal lined into two globular structure. These structure in particular are concerned with the reception and the coordination of optic inputs. The zebrafish eye is similar to the other vertebrae, it consist of three layers, first layer is the tunica fibrosa which encompasses the cornea and sclera, second layer is tunica vasculosa which encompasses the choroid and the choid rate ad iris, the third layer is retina

Inner Ear fish do not have an outer or middle ear. The vertebrate inner ear is an organ of extra ordinary functional characteristics, inner ear develops from a cranial ectoderm thickening, the otic placode visible from mid somatic stage, this placode cavities to form a hollow ball of the otic vesicle from which all structure of the membrane and the neurons of the statoacoustic ganglion are thought to arise. Five sensory patches develops in the embryonic and larval ear three cristae and two maculae.

The cardiovascular system of zebrafish

Zebrafish are prototype vertebrate, Heart of the zebrafish situated anterior of the main body cavity and ventral to the oesophagus with only single atrium and ventricle, the zebra fish heart consist of four chambers are sinus venous, atrium, ventricle, bulbus arteriosus. The heart rate of 72hpf zebrafish is around 120-180bpm.

Peripheral blood of zebrafish consist a erythrocytes/ red blood, leucocytes/ white blood cells and thrombocytes/ platelets. Erythrocytes are the main vehicle of oxygen transport and to lesser extent of carbon dioxide, they are disc shape cells with a relatively large nucleus in the Centre, shape of nucleus of erythrocyte are ellipsoid or spherical nucleus and the cytoplasm appear to be homogeneous. Leucocytes as in human plays important role in against both infectious disease and foreign material, leucocytes are divided into granulocytes and agranulocytes. Granulocytes cleavage into neutrophils and eosinophils, neutrophils which are heterophilic granulocytes, most abundant, multiplied and having segmented nucleus, eosinophils are small, non-segmented and peripherally located nucleus. Agranulocytes cleavage into lymphocytes and monocytes. Lymphocytes form about 71-92% of the circulating leucocytes population in the zebra fish they are present in the circulating blood lymph system, thymus, kidney and spleen, fish have the functional equivalent of B and T lymphocytes. Monocytes

Blood flow in the heart of zebrafish, blood flows from sinus venosus into an atrium then blood moves through the ventricles to the aorta the valves direct flow after specialized endothelium musculature drives a high pressure system then an electrical system regulates heart rhythm and pacemaker is associated with heart beat. Blood volume of zebrafish is small compared to other vertebrates, the total blood volume fish can range from 1.5 to 3.0% of total body weight, even up to 5% of body weight adult zebra fish contains ranges from 1 to 10 microliters.

The respiratory system of zebrafish

The respiratory organ of zebafish the gills are located into branchial chambers the lie on each side of the body behind the eyes. Respiration of fish is achived by bringing water through lips into the buccal cavity and hence into the pharynx and over the gills. Gill respiration only function effectively when water moves into unilateral direction over the gills members by alternately relaxing and contracting the buccal chamber and the opercula chamber. A second method for achieving water flow is by swimming with the mouth open forcing water through the pharynx over the gills and out the opercula chamber and gills slit. The gills are the structure that allow fish to efficiently extract oxygen from the water for use in metabolic reaction. The zebrafish gill are composed of four bilateral gill arches. The gill arches are supported by bony and cartilaginous tissue and contain skeletal muscle. They are richly inverted by facial, glossopharyngeal and valgus cranial nerve. They are covered by a mucinous epithelium continuous with that of the oropharynx. Extending from each gill arch are two paired primary gill filament are supported cartilage, sometime effected to as cartilage ray. Perpendicular each primary gill filament are many thin wall secondary gill filament. The secondary gill filament provides a large surface area and are the site for oxygen and ammonia. Red blood cells are easily seen in the secondary gill filament.

The digestive system of Zebrafish

Zebrafish can be fed with dry food size ranges from 300 to 400 microns or live food (brine shrimps), brine shrimps eggs (Artemis sp). Food can be intake by mouth of zebra fish, digestion of food from mouth to anus follows the following path

Digestive system of zebrafish can be divided into anterior and posterior portion. Anterior part consist mouth, buccal cavity and or pharynx. Posterior part consist esophagus, intestinal bulb, mid intestine, posterior intestine and anus. The oropharynx continues from the mouth to just posterior to the pharyngeal teeth. The esophagus continues caudally and empties into the intestinal bulb. Zebra fish are not thought to have true stomachs and the intestinal bulb is a dilated portion of the intestine, located between the esophagus and mid intestine. From the intestinal bulb, the digestive tract continues to the mid intestine, then to the posterior intestine and ends at the anus. Absorption of foods is occur in the intestinal bulb, absorption of protein occurs in the mid intestine. In the posterior intestine occurs osmoregulation

The reproductive system of Zebrafish

Reproduction in the female Zebrafish lie in the ovaries, a pair of bilateral ovaris is observed in female and it is located swim bladder and abdominal wall, across vertebrae ovaries are the site of development and production of female gametes. There are four stages of ovarian development in zebrafish. A) Primary oocyte stage with observation of relatively small spherical cells. B) Cortical alveloa stage with enlarged oocytes filled with cortical alveoli. C) Vitellogenic stage characterized by presence of egg yolk oocytes. D) Maturation stage in which oocytes with irregular layer can be observed.

Reproduction in the male zebrafish have tubule organization with in each tubule the wall lined by steroli cell. Spermatogenesis is a complex and highly coordinated process by which diploid spermatogonia produce millions of spermatozoa daily. Morphogenesis and spermatogenesis while leading cells detected in the intestinal spaces act as testosterone

Four stage of development of zebrafish from embryo to adult

Embryo: - The fertilized eggs develop rapidly with in 24hr all the major organs and tissue have formed with in the transparent embryos in three days. Locomotors behavior begins with spontaneous muscle contraction from 18hr post fertilization. Larvae: - after hatching the larva associate with hard surface by means of small secretory cells in the epidermis of the head. Juvenile: - from around 14 to 29dpf, larvae undergo a metamorphosis into the juvenile form during which time larval feature are lost and juvenile features are acquired. Adult: - at around 3 months, the juvenile become sexually mature adult and spawhing occurs. The duration of life stage is variable and dependent upon factors such as temperature, food availability.

The zebrafish genetics:-

The zebrafish genome is organized into 25 chromosomes pair. The zebrafish genetics is within the typical range for actinopterygian (ray finned) fishes, which show remarkable, conservation of chromosomes numbers the majority of teleost, have 48 or 50 chromosomes as diploid. Zebra fish has predominantly, submetacentric and metacentric chromosome. Genetics investigation of laboratory or pet store zebra fish strains have generally found no evidence of wild zebrafish although one study of wild zebra fish from northwest India reported heteromorphic, sex chromosomes with female (WZ) having submetacentric Z chromosome and metacentric W chromosomes, male (ZZ) having two submetacentric Z chromosomes.

Sex determination in zebra fish, it is unlikely that zebrafish has an established environmental sex determination mechanism (ESD) like some fish and some sauropsida (reptiles). Because male and female zebra fish develop in tanks maintained at constant temperature and single mating show that zebra fish sex determination has a strong genetic basis. Environmental factors can strongly affect the sex ratio in zebra fish, harmful factors like gamma rays, hyoxia, high temperature, high density, altered thermocycles and poor nutrition.

The excretory system of Zebrafish

Zebrafish kidney lies at retroperitoneal location, just ventral of the vertebral column. It has distinct head and trunk region. Similar to the mammalian kidney, it has nephrons with glomerulus, proximal tubule, and distal tubule. In the typical adult zebrafish at around 6 months of age this mesonephric kidney is estimated to passes approximately 450 nephrons, pronephros comprised to two nephrons. Pronephros shown to be comparible to the human metaphors. The pronephric duct is lined by epithelium from the glomerulus to the cloche, which is divided into segments comparable to mammalian system, such as proximal and the early distal tubule. The kidney functions are, given the importance of the kidney for water secretion, the presence of oedema in a mutant fish can be indicator of impared kidney function. Normal renal function tests routinely used in clinics are challenging to perform in zebrafish, due to small volume of urine, and the fact that urine is released into surrounding water.

ZEBRAFISH AS A MODEL IN HUMAN DISEASE CANCER

Cancer, zebrafish was first used in cancer research during 1960, Stanton et al. Used to test the effect of carcinogen. When exposed to carcinogenic agent such as DENA (diehtylnitrosamine) the fish will develop cancer furthermore, zebrafish has proven to be an ideal to study the malignancy of several tumors by mean tumor transplantation assays. Xenotransplantation of human tumour cell into zebrafish embryos (xenografts) it is possible to address tumor cell, migration, metastasis, angiogenesis and potential therapeutic target.

Cancer model establishment in zebrafish, using a combination of chemical treatment genetic technology and tumor cell xenotransplantation. Several carcinogenic compound are able to induce canceration in no of organ such as dimethylbenzaanthracene (DMBA), N-ethyl-N-ntrosourea (ENU) (24), Methylnitrosoguanide (MNNG). Several type of tumors have been generated by inducing mutant in known tumor suppressor gene. The knockout ps3 gene in zebrafish for example found to result in an increase of malignant peripheral nerve sheath tumor suppressor (MPNST). Several other seen mutant were found to be related to different type of tumor in zebrafish e.g.:- Nf9 gene. Through the transgenic expression of human or mouse once gene several cancer model have been established in zebrafish. T cell acute lymphoblastic leukemia was 1st cancer induced by transgenic technology in zebrafish. Which was induced by the induced by the MYC transgenes. Subsequently over expression of the oncogenes xmrk, myc and KRAS in zebrafish was found lead to hepatoma formation in both juvenile and adult transgenic fish. Xenotransplantation represents novel to established tumor model in zebrafish. The first human xenotransplant assays in zebrafish began in 2005. By injecting 1to 100 melanoma cells into the 3.5 to 4.5 HPF embryos, the migration in developing in the developing larvae was clearly observed.

Zebrafish as a tool in human cancer xenotransplantation studies could overcome some of drawbacks of the murine model. The main benefits of zebrafish are most prominent when using embryonal stage for xenotransplantation. The powerful reason for the utilization of zebrafish as pre-clinical screening model which could lead to patient-derived cancer cell xenotransplantation and to new option for personalized medicine. Transgenesis, has been traditional approach to modeling human malignancy in zebrafish, but more recently xenotransplantation of human cancer cell lines and primary human tumor sample has succefully and to study human in vitro. Xenotransplantation involve the transfer of one species tissue to another animal species and has been used as a tool for many year to cancer. The zebrafish xenotransplantation model can also be applied to drug screening and rival transgenic approaches by allowing the direct evaluation of the most clinically relevant tissue namely human cell in on 1 sample. An additional methodology to established cancer model involves transplantation of human cancer into zebrafish embryos. Zebrafish lack an innate immune system until 72 hours post fertilization and mature apaptive immune response until 4 weeks of life. Human cancer lines, purified subpopulation of cancer or primary patient system cells can be directly injected into zebrafish embryos to study many aspects of tumor biology, such as vasculature remodeling, cancer invasion and metastasis. Multiple types of human cancer cell lines and primary patient sample, including GIT, neuroendocrine, leukemic, and micrometastasis of primary tumor sample, have been successfully transplanted into 48hpf, zebrafish embryos and demonstrated their useful as new drug screening and therapeutics testing of candidate cancer drug. Invasiveness and micrometastasis of primary tumor occur within 24hr of transplantation. There zebrafish xenografts model of human cancer are especially useful in drug screening allow in for the stimulation examination of in vivo efficacy and toxicity of condition drug. Finally the advent of pigmentless Casper adult fish has enable visualization of tumor cell proliferation and dissemination in transplanted recipient beyond zebrafish embryonic stages. Adult zebrafish have three distinct classes of pigment cell black melanophore, black iridophores and yellow xanthophores. Nacre mutant zebra fish lack melanocytes, while Roy Orbison zebrafish lack iridophores. Casper zebrafish are double mutant for nacre and Roy lacking both melanocytes and iridophores through embryogenesis and adulthood. Casper permits all organs to be seen with stereomicroscopy.

Limitation of zebrafish xenotransplantation platform for drug screening, the lack of an adaptive immune response is beneficial for initial transplantation and injection it might become a limitation to translation of finding because adaptive immune cell can play vital roles in promoting or inhibiting the progression of human cancer and effect of cancer treatment.

The pathological mechanism underlying cancer are some of the most challenging processes to understand because of their variety and complexity. Zebrafish considered as complementary model to murine and other previous models for the study of genetic basis of cancer and for the evaluation of carcinogenic and antitumoral compounds in drug discovery. Zebrafish has proven to be good model to predict adverse drug effects during animal preclinical and human clinical data. The publication of the DNA sequence of zebrafish genome confirmed that relevant molecular pathway, including their implicated in cancer are similar to those of mammals, which made zebrafish an attractive choice for cancer research. A parallel approach for modeling cancer has been the (xeno) transplant of human cancer cell into zebrafish embryos, which led to the development of the so-called xenografted embryos. Zebrafish cancer model have been used for novel drug screening as well as for reanalysis known drug.

ADVANTAGES OF ZEBRAFISH AS A MODEL SYSTEM:

1. Zebrafish used in model system mainly because of their small size, big value, the main benefits of using zebrafish as a toxicological model over other vertebrate species are with regards to their size, husbandry and early morphology and financial easily availability.
2. The important advantage model system that is manifested in many elements of high-throughput chemical screening is economy funds, time and space.

3. The current and growing popularity of using zebrafish for research into human disease can be attributed to its favorable physical characteristics, ready experimental manipulation and the extensive knowledge based on this organism that now exist.
4. Zebrafish are commonly used to the study early stages of development moreover, embryonic development is quick, they are well suited for embryonic study because of their relatively transparent body during the larval development stage, and the embryos develop outside the body where they can be easily observed.
5. Genetic similarities between zebrafish and humans comparable responses to chemical treatment between zebrafish mammalian models provide a promising translation of new identified therapeutic compounds into the clinic.
6. Zebrafish emerged as powerful model for drug discovery and biosafety study because of they develop most of organ found in mammals including those of nervous, digestive, reproductive, immune, execretory and cardiovascular system
7. The transparent body of zebrafish is conducive to long term evolution of cancer cell and environmental response to them, including angiogenesis and inflammation. Cancer cells can be marked and transplanted into the zebrafish.
8. Multiple cancer models have been generated in zebrafish and proven to their human counterpart molecular and pathologically, providing excellent tool for anticancer drug discovery thorough large scale screens, candidate drug testing and target identification.

FUTURE PROSPECT:
In coming years, the increasing growth of zebrafish for biomedical research. Our data is suggest that the morphology and organ system of zebrafish is approximately similar to the mammalian organ system. Such as preclinical study of zebrafish for human diseases is easy.

Such data is necessary for the translation of results of zebrafish testing towards application in human disease treatment.

II. Conclusion:

This Review focus on the morphology of zebrafish and it's used in cancer as bio model. Hence it will be beneficial for preclinical study.

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