We are IntechOpen,
the world's leading publisher of
Open Access books
Built by scientists, for scientists

6,900
Open access books available

186,000
International authors and editors

200M
Downloads

Our authors are among the

154
Countries delivered to

TOP 1%
most cited scientists

12.2%
Contributors from top 500 universities

CLARIVATE ANALYTICS
BOOK CITATION INDEX
INDEXED

WEB OF SCIENCE™

Selection of our books indexed in the Book Citation Index
in Web of Science™ Core Collection (BKCI)

Interested in publishing with us?
Contact book.department@intechopen.com

Zebrafish (*Danio rerio*) as a Model Organism

Farmanur Rahman Khan and Saleh Sulaiman Alhewairini

Abstract

Animals as model organisms, the silent sentinels, stand watch over the environmental health of the world. These are non-human animal species which can be used to understand specific biological processes and to obtain informations which can provide an insight into working of other organisms. Among the model organisms, the zebrafish (*Danio rerio*) is one of the best leading models to study developmental biology, cancer, toxicology, drug discovery, and molecular genetics. In addition, the zebrafish is increasingly used as a genetic model organism for aquaculture species and in toxicogenomics and also to generate zebrafish disease models for application in human biomedicines. This tiny fish is a versatile model organism for many fields of research because of its easy maintenance, breeding, and transparent body during early development.

Keywords: model organisms, zebrafish, biological process, developmental biology, cancer, toxicology, drug discovery

1. Introduction

Zebrafish (*Danio rerio*) is a prominent model organism in biological researches in recent times. Zebrafish is a tropical freshwater fish, inhabitant of rivers (Ganges mainly) of Himalayan region of South Asia especially India, Nepal, Bhutan, Pakistan, Bangladesh, and Myanmar. It is a bony fish (teleost) that belongs to the family Cyprinidae under the class Actinopterygii (ray-finned fishes).

Zebrafish was first used as a biological model by George Streisinger (University of Oregon) in the 1970s because it was simpler over mouse and easy to manipulate genetically. Streisinger's colleagues especially Chuck Kimmel in his university got much impressed by the idea of using zebrafish embryo more attractive to study the development of nervous system.

The use of zebrafish as a model organism got impetus from the 1990s when it was used to develop two large genetic mutants, one by Nobel Prize winner Christiane Nusslein-Volhard in Tubingen, Germany, and the other by Wolfgang Driever and Mark Fishman in Boston, USA. The identification of mutants is one of the most important strategies for the study in various areas of biology.

Zebrafish has a lot of physiological and genetic similarities with humans, including the brain, digestive tract, musculature, vasculature, and innate immune system [1–7]. Also 70% of human disease genes have functional similarities with those of zebrafish [8].

1.1 Salient features of zebrafish as a model organism

D. Rario is preferred by scientists because of its variety of features that make it useful as a model organism. The embryo develops rapidly outside mother and optically clear and thus, easily accessible for experimentation and observation. The embryo develops very fast, and the blastula stage lasts only for 3 h, while gastrulation gets completed in 5 h; in an embryo that is about 18 h old, very well developed ears, eyes, segmenting muscles, and brain can be viewed as the embryo is transparent. By 24 h, segmentation gets completed, and most primary organ systems are formed. By 72 h, the embryo hatches out from the eggshell and within the next 2 days starts hunting for food. In a period of just 4 days, the embryo converts rapidly into a small version of adult. The rapid development simplifies development and genetic studies.

The adult zebrafish attains sexual maturity very quickly, having generation time of about 10 weeks, and also this tiny fish has good fecundity rate. When kept under optimal conditions, the zebrafish can lay about 200 eggs per week [9, 10]. Under laboratory conditions the zebrafish can spawn throughout the year that ensures the constant supply of offspring from designated pairs that makes this transparent fish a quintessential choice for large-scale genetic approaches to identify novel genes and to discover their specific functions in vertebrates [11]. The zebrafish is a very hard fish and is very easy to raise.

In addition to the features of zebrafish mentioned above, it requires very low space and maintenance cost. These features make this fish an attractive model organism for developmental, toxicological, and transgenic studies [12].

In this chapter author summarizes some of the recent advances in the area of zebrafish research, viz., developmental biology, toxicology, transgenic studies, human disease, drug discovery, cancer, etc. This review is by no means a comprehensive one but an attempt to provide a flavor to the readers some recent advances about this wonderful creature to use in potential researches.

2. Use of zebrafish in developmental biology

Much of the pioneer works that established zebrafish as a model organism were done by George Streisinger, Charles Kimmel, and their colleagues [13]. The team of these researchers studied the embryonic axis, cell lineage analysis, embryonic formation, development of central and peripheral nervous systems, muscle development, differential regulation of gene expression, etc. [14–16].

Many of the critical pathways that control development in vertebrates are highly conserved between human and zebrafish. The zebrafish genome shares a lot of similarities with human genome. About 70% of genes associated with disease in humans have functional homologs in zebrafish [8]. Realizing the importance of zebrafish model, Grunwald and Eisen used this developmental model to study the segmental structure of the brain and characterized neurons in zebrafish for the first time in a vertebrate model [17]. Nüsslein-Volhard recognized the importance of zebrafish as a vertebrate model to study developmental biology by identifying developmentally important genes [18]. The zebrafish model has been used to see the development of various systems/processes as follows.

2.1 Development of the enteric nervous system

Recently advances have been made to study the development of the enteric nervous system (ENS) using the zebrafish model. Like other vertebrates, the zebrafish

gastrointestinal tract is a complex organ composed of multiple cell types like epithelial, muscular, vascular, neural, and immune cells. The gut of the zebrafish (teleost) and amniotes have structural similarities, but in teleost it is less complex as compared to amniotes [19]. The zebrafish GI tract has no distinct stomach but an enlarged area of the anterior intestine that is known as the intestinal bulb. This intestinal bulb displays patterns of motility as well as goblet cells that produce acid and neutral mucins like the stomach of mammals [20, 21]. The gut epithelium of zebrafish is simpler than that of amniotes; it lacks crypts and is arranged in an irregular broad fold rather than forming villi [21, 22]. The genes (*sox2*, *barx1*, *gata5*, *and gata6*) which are responsible for the formation of the stomach of zebrafish also resemble with that of amniotes [23].

Like that of all vertebrates, the enteric nervous system of zebrafish is also derived from neural crest [24], but it differs potentially from amniotes wherein the enteric nervous system is derived from both the vagal and sacral crests, while in the case of zebrafish, it is derived from the vagal crest only [25–28]. Enteric neural crest cell (ENCC) migration along the gut in zebrafish is also similar with that in amniotes. It takes place in two parallel chains along the length of the developing gut [25, 27, 28]. Afterward the precursors of the ENS voyage circumferentially around the gut and differentiate into the enteric neuron and glia. The final organization of the zebrafish ENS is also very simple as compared to that of amniotes; it is composed of single neuron or small group of neurons rather than more complex ganglionated myenteric and submucosal plexuses [27, 29].

2.2 Angiogenesis

Zebrafish model also has been used in the study of angiogenesis and regeneration. Angiogenesis is the process through which new blood vessels originate from preexisting vascular structures which play essential role in healthy physiological and pathological conditions. It is achieved through interaction between endothelial cells and their niche. Inadequate maintenance leads to the development of many disorders like tissue ischemia, inflammatory disorders, retinopathies, excessive vascular growth, or abnormal remodeling that promotes cancer [30].

Being a transparent vertebrate, the zebrafish has emerged as a convenient alternative to study the early development of the cardiovascular system and observe the flow of blood [31]. In zebrafish larvae the vessels and blood flow can easily be visualized by using simple dissecting microscope and also by using fluorescent proteins; the development of the blood vascular system could be examined in great details. By using confocal microscopy and time-lapse imaging, the detailed morphogenetic movements and cell shape changes can be carried out in live specimens [31].

Vascular anatomy development of zebrafish using molecular tracers during early embryonic stages has high level of similarities with other vertebrates [1, 32, 33]. In one of the experiments, the injected fluorescent microsphere was detected when lumenization and anastomosis of the vascular network were complete [34]. The same approach was adopted to compare the development of blood and lymphatic vasculatures in zebrafish [35]. The individual cell growth during vascular development also can be tracked.

For vascular development and growth, angiogenesis plays a very important role. During embryonic development, the intersegmental vessels are formed by angiogenic sprouting from the dorsal aorta, and they have been the target of studies using genetic perturbations or drugs [36]. It has been reported that mammalian malignant cells can be xenotransplanted into zebrafish embryos, and they can form tumors [37], and thus models for tumor angiogenesis have been developed [38].

2.3 Regeneration

The zebrafish exhibits remarkable capacity of regeneration even in adult stages. The caudal fin especially provides an ideal tissue for vascular regeneration studies due to its simple and fine architecture and relative transparency [39]. After successive amputations the full regeneration of the caudal fin used to take place within couple of weeks [40]. The regenerating vessels in the regenerating caudal fin originate from vein-derived cells that have angiogenic potential [41]. These cells migrate individually or in groups and assemble into the vessel in response to chemokine signaling [42].

The zebrafish as an alternative model for angiogenesis and regeneration studies provides the relevance of in vivo assays with simplicity and versatility of in vitro assays. In larvae, access to developing vasculature through fluorophore-tagged strains and small size of zebrafish makes the use of high-throughput strategies possible. In adults, the caudal fin is equally convenient as a model tissue as regenerating vessels can be observed at all stages, and the animals (zebrafish) are suitable for experimental drug manipulations [31].

3. Zebrafish as a cancer model system

Cancer is a cursed reality for millions of humans worldwide and in fact for all vertebrates. The invertebrates such as flies and nematodes also can develop anomalies in cell proliferation. Clinically and pathologically this dreaded disease is present almost exclusively in all vertebrates, from fish to humans. To understand better the formation, growth, and spread of malignant tumors, vertebrate models are imperative. Being a vertebrate the zebrafish is an ideal model to study cancer, though humans and fishes are separated from their common ancestry but biology of the cancer in both groups of organisms is the same [43]. Because of the variety of benefits to use zebrafish as a model organism which are mentioned in "Introduction," the zebrafish is adroitly exploited to carcinogenic treatment, transplantation of mammalian tumor cells, and transgenic regulations [44].

3.1 Zebrafish as a model for carcinogen effects and development of cancer studies

Fishes are exposed to many waterborne carcinogens in the wild that lead to the development of a variety of benign and malignant tumors in teleosts, and these tumors have similar histology as in humans [45, 46]. As like humans, cancer is a genetic disease in fishes as shown by melanomas which develop in *Xiphophorus* hybrids [47]. Choosing zebrafish for modeling cancer studies has many advantages. Highly conserved cancer pathways can be screened genetically using zebrafish. Primarily cancer is a disease of adults, but through mutagenesis screens, cell cycle phenotype could be examined in rapidly developing transparent embryos of the zebrafish. The genes regulating cell cycle, cell proliferation, and apoptosis have already been screened in yeast, *Drosophila* and *C. elegans*, in the similar way gene functions for these biological pathways can be screened in zebrafish to understand the events that lead to the development of cancer in any vertebrate species [43].

By inducing different gene mutations or stimulating signaling pathways through chemicals, the tumors can be induced in different organs of the zebrafish like the pancreas, liver, GI tract, vasculature, muscles, skin, and testes [46, 48–51]. It is

possible to identify the interacting oncogenes via suppressor and enhancer screens which cause the formation of specific type of tumor. The mammalian tumor cells can be transplanted into the zebrafish, dispensing a novel way to study the interactions between transplanted tumor cell and vasculature of host.

3.2 Tumorigenesis

Tumorigenesis is a multistep process induced by chemical carcinogen [52], with accumulation of both epigenetic aberrations and mutations in regulatory regions of genes and disorder of signaling pathways [53, 54]. Methylation of DNA at CpG dinucleotides is an important component of epigenetic gene expression regulation [55] that causes the modulation of protein-DNA interactions [56, 57]. Aberrant methylation of CpG islands (CGT) takes place in the exonic and promoter regions [58, 59] and changes in gene expression associated with tumorigenesis. Hypermethylation of tumorigenetic genes has negative impact (regulation) over tumor suppressor genes (TSGs), DNA repair genes, and antiangiogenic genes, and it is a common quality of neoplastic cells [55, 60–63].

A variety of fishes have been used as model to study tumors induced by environmental carcinogens. Among all the zebrafish proved best for investigating embryogenesis, organogenesis, and impact of environmental carcinogen for the development of cancer [64]. Chemically induced tumors in zebrafish and humans are histopathologically similar [43, 65], and orthologous oncogenes and tumor suppressor genes (TSGs) have been identified in fishes and humans [65]. Hepatic gene expression in humans and zebrafish has revealed conservation of gene expression profiles at different stages of tumor aggressiveness between these two phylogenetically distant species [66, 67].

3.3 Xenotransplantation

Xenotransplantation represents another novel way to induce tumor in zebrafish. The most important feature of xenotransplantation is that tumor cells can be stained/marked by fluorescent stain that distinguishes transplanted cells from normal cells and helps in clear observation of developmental process of the tumor [68]. Several other types of tumor, such as pancreatic cancer, lung cancer, ovarian carcinoma, breast cancer, prostate cancer, retinoblastoma, leukemia, etc., have also been transplanted in the zebrafish [7].

3.4 Angiogenesis

Angiogenesis is the most important factor in tumor growth and subsequent metastasis. The importance of angiogenesis has been discussed well in the previous section on development. The vascular network is helpful to transport oxygen and nutrients to the cells; likewise tumor cells also get the supply of all these materials. Because of this reason, the development and the capability of the formation of blood vessels within the tumor determine the malignancy of the cancer as well as influence the therapeutic effects and prognosis. The endothelial cells of the vascular system can be stained by fluorescent dye/protein that helps to visualize the neovascularization of tiny tumor at the earliest stage, and metastasizing tumor cells can be tracked explicitly at cellular level [7]. The vascular system of tumor has always been the target of antitumor therapies; it is evident from research and clinical observations that if angiogenesis inhibitors are used in combination with chemotherapy, it can improve the outcome in cancer patients [69].

3.5 Skin cancer

Skin or dermal cancers represent the most common type of cutaneous malignancy globally, which includes melanoma and carcinoma of squamous cells [70]. Melanoma is the most pernicious form of skin cancer among all types of skin cancers and has mortality rate over 80% [71, 72]. Melanoma usually develops in the pigmented epidermal cells (melanocytes), which are responsible for the production of melanin. In the beginning of melanoma, it is restricted to the epidermis because of the radial growth phase (RGP) of melanoma, and it can be removed by surgical excision [73]. In later stages of tumor progression, the melanoma cells invade the subcutaneous tissues due to vertical growth phase (VGP) of melanoma and eventually lead toward the metastatic phase. At this stage, very limited therapeutic options are available, and melanoma frequently deteriorates and becomes untreatable [73, 74].

Cutaneous squamous cell carcinoma (cSCC) mostly develops due to UV radiation exposure of epidermal cells, namely, keratinocytes, in which uncontrolled proliferation starts [75]. cSCC accounts for the most frequent type of non-melanoma cutaneous cancer and constitutes about 20% of all skin malignancies [75, 76].

SCCs are curable in situ by surgical excision. Metastatic SCCs are responsible for majority of deaths due to non-melanoma skin cancer [70]. Head and neck squamous cell carcinoma (HNSCC) develops in various places such as the oropharynx and laryngopharynx which is very common worldwide [77]. Especially oral squamous cell carcinoma (OSCC) accounts for about 24% of HNSCC with a mortality rate of 2 million deaths every year [76, 78].

Zebrafish is a powerful in vivo tool to study pathologies and treatment for skin cancer (melanoma and SCC). The zebrafish can be used to study melanoma development, progression, drug screening, and treatment. The zebrafish model has been exploited recently to recognize the key molecules which are responsible for the development of cutaneous squamous cell carcinoma (cSCC) and head and neck squamous cell carcinoma (HNSCC) [72] as well as for SCC target therapies [79].

3.6 Tumor metastasis

Metastasis is a multistep and complex process in which tumor cells penetrate in the vascular system and spread deep in parenchymatous tissues [80]. For better therapeutic practices like development of antitumor drugs and advancements of clinical treatments, the insight into mechanism of tumor metastasis is very helpful. Because of many significant disadvantages in the previous studies using in vivo mouse model, the metastasis process cannot be abstracted properly, but zebrafish cancer model has overcome the drawback of previous models and has shown exceptional strengths. The adaptive immune system in larvae of zebrafish usually develops after 14 DPF, which provides very conducive environment for survival of transplanted cancer cells and metastasis [81], and the process of tumor metastasis can be observed through the transparent body of zebrafish under microscope. To better understand the process of metastasis, the transplanted tumor cells can be stained/treated by dye like CM-Dil or may be labeled by red fluorescent protein (RFP) [82]. Mammalian tumor cells treated with red fluorescent protein when injected into transgenic zebrafish, the process of tumor cell metastasis and angiogenesis can be viewed well after 48 h of transplantation [83]. By using zebrafish, the suppressing or promoting factors for metastasis can be identified. In RFP treated U87 glioma stem cells (GSCs), when transplanted into the yolk sac of the zebrafish embryo, the various invasive stages of GSCs like approaching, cluster formation, invasion, migration, and transmigration can be observed clearly at 48 h postinjection [83].

4. Toxicology and drug discovery

As discussed previously in Section 1, because of many advantages, the zebrafish has recently emerged as a prominent model for toxicological studies and drug discovery. The effects of drugs on growth and development can be examined visually through length and shape of the zebrafish body as well as the morphology of internal organs such as the brain, liver, cardiovascular system, pancreas, intestine, kidney, notochord, etc. The zebrafish model also has been used to know the organ function assays and assessment of drug effect [84].

Zebrafish embryos are used as predictive model to assess the toxicity in mammals. The lethal concentration (LC_{50}) of different chemicals has been determined in embryos of zebrafish and has been compared with the mammalian LC_{50}, and it has been found that median lethal dose of zebrafish is lower than mammals [84]. The effects of drugs on specific organs have also been studied, and it has been found that organ toxicity is similar in both zebrafish and mammals. The drugs that were used to evaluate the organ toxicity were gentamicin, cisplatin, vinblastine, quinine, neomycin, doxorubicin, dexamethasone, cyclosporin A, caffeine, camptothecin, MPA, fluorouracil, etc. [85–90].

4.1 Drug toxicity

In drug development, the toxicity plays a major role. Due to the toxicity problem, many new drugs have been declined by the FDA. The evaluation of toxicity of drug is very essential to know the end points of toxicity, dose-response relationships, and mechanism of toxicity and also to determine the toxicodynamics of the drug [91].

The zebrafish is acquiring the reputation rapidly as a promising model animal to study drug and chemical toxicology [92, 93]. The toxicity of some of the important drugs has been examined using the zebrafish model, for instance, Amanuma et al. [94] developed a test in which susceptible zebrafish was used to detect small molecule-induced mutagenesis. The embryos of zebrafish were utilized to compare the developmental toxicity resulting from the exposure to ethanol or acetaldehyde [95]. Toxicity of antirheumatic drug like diclofenac was evaluated by using zebrafish. Now, zebrafish has got the status of a successful animal model to study drug toxicity and toxicology caused by environmental contaminants [91].

4.2 Zebrafish and drug discovery

The zebrafish model has been used potentially in drug discovery and to know the effects of neurotoxic, ototoxic, and neuroprotectant drugs. The process of drug discovery is divided into four main components: screening of lead compounds, target identification, target validation, and assay development [96]. The process of target identification involves the recognition of target gene or protein which when modulated by a drug can have positive effects on the progression of disease. After identification of possible target, the validation process of target begins through determination of protein function and assessment of the druggability of the target [84, 97–99]. Zebrafish has great role in each of these areas of drug discovery.

4.3 Angiogenesis

The angiogenesis has already been discussed earlier in detail in previous sections on development and cancer. The impact of various proangiogenic compounds like simvastatine or penicillamine[20] or antiangiogenic compounds like vandetanib

or PTK787 can be assessed well and visualized through the development of the vascular system in transparent zebrafish embryo [84].

4.4 Cardiotoxicity

In drug development, cardiotoxicity is one of the major concerns. Through the transparent zebrafish embryo, various cardiac functions like heart rate, rhythm, contraction, circulation, etc. can be assessed directly. It has been demonstrated well that toxic effects of ten cardiotoxic agents in zebrafish embryos have similar impact as in humans [100]. Treatment with terfenadine and clomipramine caused severe impairment of cardiac functions, edema, hemorrhage, arrested heartbeat, and even death. These results in zebrafish exhibit similarities with humans [101]. Another group of researchers proposed to use a transgenic model for high-throughput testing of small molecules that modulate the heart rate of the zebrafish embryo [102]. Thus, zebrafish is a suitable model for preliminary screening of molecules which have potential therapeutic or toxic effects.

5. Human disease and zebrafish

Most of the tissues and organs found in humans and zebrafish are the same except lungs and prostrate and mammary glands. The cloning of mutated genes screened for specific phenotypes in zebrafish has similarities in humans and thus serves as model for human disease and to study underlying mechanisms. The first human disease identified using zebrafish was a blood disorder involving specific defect in hemoglobin production through ALAS2 mutated gene [103].

Many other mutants which show phenotypic similarities to human disease have been screened and identified. These include neurological disorders [104], hematological disorder [105, 106], cardiovascular diseases [107], muscle disease [108] and cancers [109, 110], Parkinson's disease [111], anxiety, and posttraumatic stress disorder [112].

6. Zebrafish as a model organism for aquaculture species

Among different fish species of interest to aquaculture, zebrafish is genetically more tractable. The zebrafish model is used commercially in many areas of aquaculture such as in the identification of genes involved in the development of the muscles, bones, and fats, the metabolism of nutrients, disease, and stress pathways and also behavioral traits. The drugs which affect the physiology of the fishes can be tested easily in zebrafish especially their effect on a range of alleles to assess their genetic property [113]. Many researches have been done regarding the improvement of diet and their husbandry to improve the growth rate and reduce stress and disease in many fish species like gilthead seabream, seabass, rainbow trout, Atlantic salmon, tilapia, catfish, cod, etc. [10]. The zebrafish disease models are being used in various infections of aquaculture, for instance, tuberculosis and streptococcal and salmonella infections [114].

7. Conclusion

Zebrafish is a successful and versatile animal model system, offering a tool to model gene function, development of various organ systems, cancer studies,

toxicology, drug discovery, human disease and disorders and also in aquaculture, etc. because low cost and easy maintenance, transparent embryo, easy manipulation, high fecundity, and rapid embryonic development favor the zebrafish as an attractive model for in vivo assays with simplicity and versatility of in vitro assays over mammalian models which lack all of these benefits. The future of zebrafish as model organism is very bright. In coming years, an increased number of reports are expected on the application of zebrafish as an effective bioindicator.

Conflict of interest

The author declares that there is no conflict of interest.

Author details

Farmanur Rahman Khan[1*] and Saleh Sulaiman Alhewairini[2]

1 Department of Biology, Deanship of Educational Services, Qassim University, Al Qassim, Buraidah, Saudi Arabia

2 Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Qassim University, Al Qassim, Buraidah, Saudi Arabia

*Address all correspondence to: insectqh11@gmail.com

IntechOpen

References

[1] Gore AV, Monzo K, Cha YR, Pan W, Weinstein BM. Vascular development in the zebrafish. Cold Spring Harbor Perspectives in Medicine. 2012;**2**(5):a006684

[2] Kanungo J, Cuevas E, Ali SF, Paule MG. Zebrafish model in drug safety assessment. Current Pharmaceutical Design. 2014;**20**(34):5416-5429

[3] Kalueff AV, Stewart AM, Gerlai R. Zebrafish as an emerging model for studying complex brain disorders. Trends in Pharmacological Sciences. 2014;**35**(2):63-75

[4] Guyon JR, Steffen LS, Howell MH, Pusack TJ, Lawrence C, Kunkel LM. Modeling human muscle disease in zebrafish. Biochimica et Biophysica Acta. 2007;**1772**(2):205-215

[5] Weinstein B. Vascular cell biology in vivo: A new piscine paradigm? Trends in Cell Biology. 2002;**12**(9):439-445

[6] Lieschke GJ, Oates AC, Crowhurst MO, Ward AC, Layton JE. Morphologic and functional characterization of granulocytes and macrophages in embryonic and adult zebrafish. Blood. 2001;**98**(10):3087-3096

[7] Zhao S, Huang J, Ye J. A fresh look at zebrafish from the perspective of cancer research. Journal of Experimental & Clinical Cancer Research. 2015;**34**:80. DOI: 10.1186/s13046-015-0196-8

[8] Santoriello C, Zon LI. Hooked! Modeling human disease in zebrafish. The Journal of Clinical Investigation. 2012;**122**(7):2337-2343

[9] Brand M, Granato M, Nusslein-Volhard C. Keeping and raising zebrafish. In: Nusslein-Volhard C, Dahm R, editors. Zebrafish: A Practical Approach. Oxford: Oxford University Press; 2002. pp. 7-37

[10] Carpio Y, Estrada MP. Zebrafish as a genetic model organism. Biotecnologia Aplicada. 2006;**23**:265-270

[11] Pelegri F. Mutagenesis. In: Nusslein-Volhard C, Dahm R, editors. Zebrafish: A Practical Approach. Oxford: Oxford University Press; 2002. pp. 145-174

[12] Lele Z, Krone PH. The zebrafish as a model system in developmental, toxicological and transgenic research. Biotechnology Advances. 1996;**14**(1):57-72

[13] Kimmel CB. Genetics and early development of zebrafish. Trends in Genetics. 1989;**5**:283-288

[14] Chakrabarti S, Streisinger G, Singer F, Walker C. Frequency of gamma-ray induced specific locus and recessive lethal mutations in mature germ cells of the zebrafish, *Brachydanio rerio*. Genetics. 1983;**103**:109-123

[15] Streisinger G, Singer F, Walker C, Knauber D, Dower N. Segregation analyses and gene-centromere distances in zebrafish. Genetics. 1986;**112**:311-319

[16] Walker C, Streisinger G. Induction of mutations by gamma-rays in pregonial germ cells of zebrafish embryos. Genetics. 1983;**103**:125-136

[17] Grunwald DJ, Eisen JS. Headwaters of the zebrafish—Emergence of a new model vertebrate. Nature Reviews. Genetics. 2002;**3**:717-724

[18] Nüsslein-Volhard C, Wieschaus E. Mutations affecting segment number and polarity in Drosophila. Nature. 1980;**287**:795-801

[19] Wallace KN, Akhter S, Smith EM, Lorent K, Pack M. Intestinal growth and differentiation in zebrafish. Mechanisms of Development. 2005;**122**:157-173

[20] Holmberg A, Olsson C, Hennig GW. TTX-sensitive and TTX-insensitive control of spontaneous gut motility in the developing zebrafish (*Danio rerio*) larvae. The Journal of Experimental Biology. 2007;**210**:1084-1091

[21] Ng AN, de Jong-Curtain TA, Mawdsley DJ, White SJ, Shin J, Appel B, et al. Formation of the digestive system in zebrafish: III. Intestinal epithelium morphogenesis. Developmental Biology. 2005;**286**:114-135

[22] Wallace AS, Burns AJ. Development of the enteric nervous system, smooth muscle and interstitial cells of Cajal in the human gastrointestinal tract. Cell and Tissue Research. 2005;**319**:367-382

[23] Muncan V, Faro A, Haramis AP, Hurlstone AF, Wienholds E, van Es J, et al. T-cell factor 4 (Tcf7l2) maintains proliferative compartments in zebrafish intestine. EMBO Reports. 2007;**8**:966-973

[24] Kelsh RN, Eisen JS. The zebrafish colourless gene regulates development of non-ectomesenchymal neural crest derivatives. Development. 2000;**127**:515-525

[25] Elworthy S, Pinto JP, Pettifer A, Cancela ML, Kelsh RN. Phox2b function in the enteric nervous system is conserved in zebrafish and is sox10-dependent. Mechanisms of Development. 2005;**122**:659-669

[26] Furness JB. The Enteric Nervous System. Oxford: John Wiley and Sons Ltd.; 2006

[27] Olden T, Akhtar T, Beckman SA, Wallace KN. Differentiation of the zebrafish enteric nervous system and intestinal smooth muscle. Genesis. 2008;**46**:484-498

[28] Shepherd IT, Pietsch J, Elworthy S, Kelsh RN, Raible DW. Roles for GFRalpha1 receptors in zebrafish enteric nervous system development. Development. 2004;**131**:241-249

[29] Tiffany AH, Shepherd IT, Burns AJ. Enteric nervous system development in avian and zebrafish models. Developmental Biology. 2016;**417**(2):129-138. DOI: 10.1016/j.ydbio.2016.05.017

[30] Pandya N, Dhalla N, Santani D. Angiogenesis—A new target for future therapy. Vascular Pharmacology. 2006;**44**:265-274. DOI: 10.1016/j.vph.2006.01.005

[31] Chávez MN, Aedo G, Fierro FA, Allende ML, Egaña JT. Zebrafish as an emerging model organism to study angiogenesis in development and regeneration. Frontiers in Physiology. 2016;**7**:56. DOI: 10.3389/fphys.2016.00056

[32] Isogai S, Horiguchi M, Weinstein BM. The vascular anatomy of the developing zebrafish: An atlas of embryonic and early larval development. Developmental Biology. 2001;**230**:278-301. DOI: 10.1006/dbio.2000.9995

[33] Ellertsdóttir E, Lenard A, Blum Y, Krudewig A, Herwig L, Affolter M. Vascular morphogenesis in the zebrafish embryo. Developmental Biology. 2001;**341**:56-65. DOI: 10.1016/j.ydbio.2009.10.035

[34] Küchler AM, Gjini E, Peterson-Maduro J, Cancilla B, Wolburg H, Schulte-Merker S. Development of the zebrafish lymphatic system requires VEGFC signaling. Current Biology. 2006;**16**:1244-1248. DOI: 10.1016/j.cub.2006.05.026

[35] Coffindaffer-Wilson M, Craig MP, Hove JR. Determination of lymphatic vascular identity and developmental timecourse in zebrafish (*Danio rerio*). Lymphology. 2011;**44**:1-12

[36] Schuermann A, Helker CS, Herzog W. Angiogenesis in zebrafish. Seminars in Cell & Developmental Biology. 2014;**31**:106-114. DOI: 10.1016/j.semcdb.2014.04.037

[37] Haldi M, Ton C, Seng WL, McGrath P. Human melanoma cells transplanted into zebrafish proliferate, migrate, produce melanin, form masses and stimulate angiogenesis in zebrafish. Angiogenesis. 2006;**9**:139-151. DOI: 10.1007/s10456-006-9040-2

[38] Tobia C, De Sena G, Presta M. Zebrafish embryo, a tool to study tumor angiogenesis. The International Journal of Developmental Biology. 2011;**55**:505-509. DOI: 10.1387/ijdb.103238ct

[39] Poss KD, Keating MT, Nechiporuk A. Tales of regeneration in zebrafish. Developmental Dynamics. 2003;**226**:202-210. DOI: 10.1002/dvdy.10220

[40] Azevedo A, Grotek B, Jacinto A, Weidinger G, Saúde L, Karl M. The regenerative capacity of the zebrafish caudal fin is not affected by repeated amputations. PLoS One. 2011;**6**:e22820. DOI: 10.1371/journal.pone.0022820

[41] Xu C, Hasan SS, Schmidt I, Rocha SF, Pitulescu ME, Bussmann J. Arteries are formed by vein-derived endothelial tip cells. Nature Communications. 2014;**5**:5758. DOI: 10.1038/ncomms6758

[42] Hasan SS, Siekmann AF. The same but different: Signaling pathways in control of endothelial cell migration. Current Opinion in Cell Biology. 2015;**36**:86-92. DOI: 10.1016/j.ceb.2015.07.009

[43] Amatruda JF, Shepard JL, Stern HM, Zon LI. Zebrafish as a cancer model system. Cancer Cell. 2002;**1**:229-231

[44] Mizgirev I, Revskoy S. Generation of clonal zebrafish lines and transplantable hepatic tumors. Nature Protocols. 2010;**5**(3):383-394

[45] Hawkins WE, Overstreet RM, Fournie JW, Walker WW. Development of aquarium fish models for environmental carcinogenesis: Tumor induction in seven species. Journal of Applied Toxicology. 1985;**5**:261-264

[46] Spitsbergen JM, Tsai HW, Reddy A, Miller T, Arbogast D, Hendricks JD, et al. Neoplasia in zebrafish (*Danio rerio*) treated with 7,12-dimethylbenz[a]anthracene by two exposure routes at different developmental stages. Toxicologic Pathology. 2000;**28**:705-715

[47] Walter RB, Kazianis S. Xiphophorus interspecies hybrids as genetic models of induced neoplasia. ILAR Journal. 2001;**42**:299-321

[48] Basten SG, Davis EE, Gillis AJ, van Rooijen E, Stoop H, Babala N, et al. Mutations in LRRC50 predispose zebrafish and humans to seminomas. PLoS Genetics. 2013;**9**(4):e1003384

[49] Lam SH, Wu YL, Vega VB, Miller LD, Spitsbergen J, Tong Y, et al. Conservation of gene expression signatures between zebrafish and human liver tumors and tumor progression. Nature Biotechnology. 2006;**24**(1):73-75

[50] Mizgireuv IV, Revskoy SY. Transplantable tumor lines generated in clonal zebrafish. Cancer Research. 2006;**66**(6):3120-3125

[51] Spitsbergen JM, Tsai HW, Reddy A, Miller T, Arbogast D, Hendricks JD, et al. Neoplasia in zebrafish (*Danio rerio*) treated with N-methyl-N'-nitro-Nnitrosoguanidine by three exposure routes at different developmental stages. Toxicologic Pathology. 2000;**28**(5):716-725

[52] Counts JL, Goodman JI. Hypomethylation of DNA: A

nongenotoxic mechanism involved in tumor promotion. Toxicology Letters. 1995;**82/83**:663-672

[53] Tischoff I, Tannapfel A. DNA methylation in hepatocellular carcinoma. World Journal of Gastroenterology. 2008;**14**(11):1741-1748

[54] Gronbaek K, Hother C, Jones PA. Epigenetic changes in cancer. APMIS. 2007;**115**:1039-1059

[55] Rauch T, Wang Z, Zhang X, Zhong X, Wu X, Lau SK, et al. Homeobox gene methylation in lung cancer studied by genome-wide analysis with microarray-based methylation CpG island recovery assay. PNAS. 2007;**104**(13):5527-5532

[56] Jones PA, Takai D. The role of DNA methylation in mammalian epigenetics. Science. 2001;**293**:1068-1070

[57] Pomraning KR, Smith KM, Freitag M. Genome-wide high throughput analysis of DNA methylation in eukaryotes. Methods. 2009;**47**:142-150

[58] Weber M, Davies JJ, Wittig D, Oakeley EJ, Haase M, Lam WL, et al. Chromosome-wide and promoter-specific analyses identify sites of different DNA methylation in normal and transformed human cells. Nature Genetics. 2005;**37**(8):853-862

[59] Takai D, Jones PA. Comprehensive analysis of CpG islands in human chromosome 21 and 22. PNAS. 2002;**99**(6):3740-3745

[60] Rauch T, Li H, Wu X, Pfeifer GP. MIRA-assisted microarray analysis, a new technology for the determination of DNA methylation patterns, identifies frequent methylation of homeodomain-containing genes in lung cancer cells. Cancer Research. 2006;**66**(16):7939-7947

[61] Lopez J, Percharde M, Coley HM, Webb A, Crook T. The context and potential of epigenetics in oncology. British Journal of Cancer. 2009;**100**:571-577

[62] Momparler RL, Bovenzi V. DNA methylation and cancer. Journal of Cellular Physiology. 2000;**183**:145-154

[63] Baylin SB, Herman JG. DNA hypermethylation in tumourigenesis. Epigenetics joins genetics. Trends in Genetics. 2000;**16**(4):168-174

[64] Bailey GS, Williams DE, Hendricks JD. Fish models for environmental carcinogenesis: The rainbow trout. Environmental Health Perspectives. 1996;**104**(1):5-21

[65] Berghmans S, Jette C, Langenau D, Hsu K, Stewart R, Look T, et al. Making waves in cancer research: New models in the zebrafish. BioTechniques. 2005;**39**:227-237

[66] Larn S, Wu Y, Vega VB, Miller LD, Spitsbergen J, Tong Y, et al. Conservation of gene expression signature between zebrafish and human liver tumors and tumor progression. Nature Biotechnology. 2006;**24**(1):73-75

[67] Mirbahai L, Williams T, Zhan H, Gong Z, Chipman JK. Comprehensive profiling of zebrafish hepatic proximal promoter CpG island methylation and its modification during chemical carcinogenesis. BMC Genomics. 2011;**12**:3

[68] Smith AC, Raimondi AR, Salthouse CD, Ignatius MS, Blackburn JS, Mizgirev IV, et al. High-throughput cell transplantation establishes that tumorinitiating cells are abundant in zebrafish T-cell acute lymphoblastic leukemia. Blood. 2010;**115**(16):3296-3303

[69] Bellou S, Pentheroudakis G, Murphy C, Fotsis T. Anti-angiogenesis in cancer therapy: Hercules and hydra. Cancer Letters. 2013;**338**(2):219-228

[70] Von Massenhausen A, Sanders C, Bragelmann J, Konantz M, Queisser A, Vogel W, et al. Targeting DDR2 in head and neck squamous cell carcinoma with dasatinib. International Journal of Cancer. 2016;**139**:2359-2369

[71] Ochoa-Alvarez JA, Krishnan H, Pastorino JG, Nevel E, Kephart D, Lee JJ, et al. Antibody and lectin target podoplanin to inhibit oral squamous carcinoma cell migration and viability by distinct mechanisms. Oncotarget. 2016;**6**:9045-9060

[72] Shin YS, Cha HY, Lee BS, Kang SU, Hwang HS, Kwon HC, et al. Anti-cancer effect of luminacin, a marine microbial extract, in head and neck squamous cell carcinoma progression via autophagic cell death. Cancer Research and Treatment. 2016;**48**:738-752

[73] Michailidou C, Jones M, Walker P, Kamarashev J, Kelly A, Hurlstone AF. Dissecting the roles of Raf and PI3K-signalling pathways in melanoma formation and progression in a zebrafish model. Disease Models & Mechanisms. 2009;**2**:399-411

[74] Fernandez Del Ama L, Jones M, Walker P, Chapman A, Braun JA, Mohr J, et al. Reprofiling using a zebrafish melanoma model reveals drugs cooperating with targeted therapeutics. Oncotarget. 2016;**7**:40348-40361

[75] Martins VL, Caley MP, Moore K, Szentpetery Z, Marsh ST, Murrell DF, et al. Suppression of TGFβ and angiogenesis by type VII collagen in cutaneous SCC. Journal of the National Cancer Institute. 2015;**108**(1):djv293. https://doi.org/10.1093/jnci/djv293

[76] Xiong P, Xiao LY, Yang R, Guo Q, Zhao YQ, Li W, et al. Flotillin-1 promotes cell growth and metastasis in oral squamous cell carcinoma. Neoplasma. 2013;**60**:395-405

[77] Bootorabi F, Manouchehri H, Changizi R, Barker H, Palazzo E, Saltari A, et al. Zebrafish as a model organism for the development of drugs for skin cancer. International Journal of Molecular Sciences. 2017;**18**:E1550

[78] Xiong P, Li YX, Tang YT, Chen HG. Proteomic analyses of Sirt1-mediated cisplatin resistance in OSCC cell line. The Protein Journal. 2011;**30**:499-508

[79] Jung DW, Kim J, Che ZM, Oh ES, Kim G, Eom SH, et al. A triazine compound S06 inhibits proinvasive crosstalk between carcinoma cells and stromal fibroblasts via binding to heat shock protein 90. Chemistry & Biology. 2011;**18**:1581-1590

[80] Eccles SA, Welch DR. Metastasis: Recent discoveries and novel treatment strategies. Lancet. 2007;**369**(9574):1742-1757

[81] Traver D, Herbomel P, Patton EE, Murphey RD, Yoder JA, Litman GW, et al. The zebrafish as a model organism to study development of the immune system. Advances in Immunology. 2003;**81**:253-330

[82] Marques IJ, Weiss FU, Vlecken DH, Nitsche C, Bakkers J, Lagendijk AK, et al. Metastatic behaviour of primary human tumours in a zebrafish xenotransplantation model. BMC Cancer. 2009;**9**:128

[83] Yang XJ et al. A novel zebrafish xenotransplantation model for study of glioma stem cell invasion. PLoS One. 2013;**8**(4):e61801

[84] Kari G, Rodeck U, Dicker AP. Zebrafish: An emerging model system for human disease and drug discovery. Clinical Pharmacology and Therapeutics. 2007;**82**(1):70-80

[85] Zhang C, Willett C, Fremgen T. Zebrafish: An animal model for toxicological studies. Current

Protocols in Toxicology. 2003. DOI: 10.1002/0471140856.tx0107s17

[86] Daroczi B, Kari G, McAleer MF, Wolf JC, Rodeck U, Dicker AP. In vivo radioprotection by the fullerene nanoparticle DF-1 as assessed in a zebrafish model. Clinical Cancer Research. 2006;**12**:7086-7091

[87] Langheinrich U, Hennen E, Stott G, Vacun G. Zebrafish as a model organism for the identification and characterization of drugs and genes affecting p53 signaling. Current Biology. 2002;**12**:2023-2028

[88] McAleer MF et al. Novel use of zebrafish as a vertebrate model to screen radiation protectors and sensitizers. International Journal of Radiation Oncology, Biology, Physics. 2005;**61**:10-13

[89] Ton C, Parng C. The use of zebrafish for assessing ototoxic and otoprotective agents. Hearing Research. 2005;**208**:79-88

[90] Wu X, Zhong H, Song J, Damoiseaux R, Yang Z, Lin S. Mycophenolic acid is a potent inhibitor of angiogenesis. Arteriosclerosis, Thrombosis, and Vascular Biology. 2006;**26**:2414-2416

[91] Chakraborty C, Hsu CH, Wen ZH, Lin CS, Agoramoorthy G. Zebrafish: A complete animal model for in vivo drug discovery and development. Current Drug Metabolism. 2009;**10**:116-124

[92] Spitsbergen JM, Kent ML. The state of the art of the zebrafish model for toxicology and toxicologic pathology research—Advantages and current limitations. Toxicologic Pathology. 2003;**31**:62-87

[93] Rubinstein AL. Zebrafish assays for drug toxicity screening. Expert Opinion on Drug Metabolism & Toxicology. 2006;**2**(2):231-240

[94] Amanuma K, Takeda H, Amanuma H, Aoki Y. Transgenic zebrafish for detecting mutations caused by compounds in aquatic environments. Nature Biotechnology. 2000;**18**(1):62-65

[95] Reimers MJ, Flockton AR, Tanguay RL. Ethanol- and acetaldehyde-mediated developmental toxicity in zebrafish. Neurotoxicology and Teratology. 2004;**26**(6):769-781

[96] Handen JS. The industrialization of drug discovery. Drug Discovery Today. 2002;**7**(2):83-85

[97] Eckstein J. ISOA/ARF drug development tutorial. http://www.alzforum.org/drg/tut/tutorial.asp

[98] Lindsay MA. Target discovery. Nature Reviews. Drug Discovery. 2003;**2**(10):831-838

[99] Frank SD. Target-based drug discovery: Is something wrong? Drug Discovery Today. 2005;**10**(2):139-147

[100] Langheinrich U, Vacun G, Wagner T. Zebrafish embryos express an orthologue of HERG and are sensitive toward a range of QT-prolonging drugs inducing severe arrhythmia. Toxicology and Applied Pharmacology. 2003;**193**:370-382

[101] Lawson ND, Weinstein BM. In vivo imaging of embryonic vascular development using transgenic zebrafish. Developmental Biology. 2002;**248**:307-318

[102] Burns CG, Milan DJ, Grande EJ, Rottbauer W, MacRae CA, Fishman MC. High-throughput assay for small molecules that modulate zebrafish embryonic heart rate. Nature Chemical Biology. 2005;**1**:263-264

[103] Chitramuthu BP. Modeling human disease and development in zebrafish. Human Genet Embryology. 2013;**3**:e108. DOI: 10.4172/2161-0436.1000e108

[104] Gama Sosa MA, De Gasperi R, Elder GA. Modeling human neurodegenerative diseases in transgenic systems. Human Genetics. 2012;**131**:535-563

[105] Berman J, Payne E, Hall C. The zebrafish as a tool to study hematopoiesis, human blood diseases, and immune function. Advances in Hematology. 2012;**2012**:2. Article ID 425345. https://doi.org/10.1155/2012/425345

[106] Brownlie A, Donovan A, Pratt SJ, Paw BH, Oates AC, et al. Positional cloning of the zebrafish sauternes gene: A model for congenital sideroblastic anaemia. Nature Genetics. 1998;**20**:244-250

[107] Sehnert AJ, Huq A, Weinstein BM, Walker C, Fishman M, et al. Cardiac troponin T is essential in sarcomere assembly and cardiac contractility. Nature Genetics. 2002;**31**:106-110

[108] Lin YY. Muscle diseases in the zebrafish. Neuromuscular Disorders. 2012;**22**:673-684

[109] Liu S, Leach SD. Zebrafish models for cancer. Annual Review of Pathology. 2011;**6**:71-93

[110] Patton EE, Widlund HR, Kutok JL, Kopani KR, Amatruda JF, et al. BRAF mutations are sufficient to promote nevi formation and cooperate with p53 in the genesis of melanoma. Current Biology. 2005;**15**:249-254

[111] Sarath Babu N, Murthy CLN, Kakara S, Sharma R, Swamy B, Cherukuvada V, et al. 1-Methyl-4-phenyl-1, 2,3, 6-tetrahydropyridine induced Parkinson's disease in zebrafish. Proteomics. 2016;**16**:1407-1420

[112] Chakravarty S, Reddy BR, Sudhakar SR, Saxena S, Das T, Meghah V, et al. Chronic unpredictable stress (CUS)-induced anxiety and related mood disorders in a zebrafish model: Altered brain proteome profile implicates mitochondrial dysfunction. PLoS One. 2013;**8**:e63302

[113] Dahm R, Geisler R. Learning from small fry: The zebrafish as a genetic model organism for aquaculture fish species. Marine Biotechnology. 2006;**8**(4):329-345

[114] Van der Sar AM, Appelmelk BJ, Vandenbroucke-Grauls CM, Bitter W. A star with stripes: Zebrafish as an infection model. Trends in Microbiology. 2004;**12**:451-457