Review Article

Zebra Fish as a Cancer Model

Sarguru Datchinamurthi*, Ilanchezhian.T, Vanaja, Balaji Rajagopalan
Department of Biochemistry, Shri Sathya Sai Medical College & Research Institute, Tamilnadu, India.
*Corresponding author's E-mail: sarguru2@gmail.com

ABSTRACT

The zebra fish has developed into an important model organism in biomedical research over the last two decades. Although the main focus of zebra fish research has habitually been on developmental biology, observing zebra fish in the lab led to the identification of diseases similar to humans, such as cancer, which subsequently became a subject for study. As a result, about more than 50 articles have been published since 2000 in which zebra fish were used as a cancer model. Strategies used include carcinogenic treatments, transplantation of mammalian cancer cells, forward genetic screens for proliferation or genomic instability, reverse genetic target-selected mutagenesis to inactivate known tumor suppressor genes, and the generation of transgenics to express human oncogenes. Zebra fish found to develop almost all type of tumor known from human, with similar morphology and, accordance to gene expression array studies, equivalent signaling pathways. However, tumor incidences were found to be relatively low, although highly comparable between different mutants, and tumors develop late in life. In addition, tumor spectra are sometimes different when compared with mice and humans. However, the zebra fish model has created its own slot in cancer research, complementing existing models with its specific experimental advantages and characteristics. Examples of these are imaging of tumor progression in living fish by fluorescence, treatment with chemical compounds, and screening possibilities not only for chemical modifiers but also for genetic enhancers and suppressors. This review aims to provide a comprehensive overview of the state of the art of zebra fish as a model in cancer research.

Keywords: Cancer, Zebra fish, tumour.

INTRODUCTION

Cancer Research in Zebra fish

Zebra fish has been used as a laboratory model for a few decades now. Originally, the main focus was on developmental biology because of the clear advantages of zebra fish such as size, transparent embryos, and ex utero development of the embryo, however, researchers found different diseases in adults, including cancer. Studies on the latter revealed that zebra fish spontaneously develop almost all type of tumor [1-4]. The most common target tissues for neoplasia are the testis, thyroid, liver, peripheral nerve, connective tissue, and ultimobranchial gland. Less common target tissues include blood vessels, brain, gill, nasal epithelium, and the lymphomyeloid system [2]. In this review the currently used approaches to induce tumor in zebra fish will be discussed.

Treatment with Mutagens

Previously, researchers appreciated the relative ease of treating fish with carcinogens because the chemicals can be dissolved in water and the animals can be exposed for longer periods. When exposing zebrafish to different compounds [e.g., 7,12-dimethylbenz(a)anthracene, N-nitrosodimethylamine, and N-nitrosodiethylamine], mainly liver and intestinal tumors were observed [5-7]. More in recent times, similarly but more extensive studies showed that N-nitrosodiethylamine primarily induces liver and pancreas carcinomas (8), and N-nitrosodimethylamine induces only liver tumors [9]. 7,12-Dimethylbenz[a]anthracene induces the broadest tumor spectrum, including liver neoplasms; epithelial tumors in intestine, pancreas, thyroid, mesenchymal tumors in cartilage, blood vessels, muscles, and connective and lymphoid tissues; and neural tumors [10]. N-Methyl-N-nitro-N-nitrosoguanidine can also induce different tumor types, mainly liver and testicular neoplasms, as well as hemangio(sarco)mas and others [11]. Some of these carcinogenic treatments have been applied to mutants with a genetic tendency to cancer but a low spontaneous tumor incidence to show an increased sensitivity of mutants compared with treated wild-type animals [12-14]. Not surprisingly, the mutagen N-ethyl-N-nitrosourea, which induces point mutations and is commonly used in forward and reverse genetic screens, Following a group of animals from a mutagenesis screen, Beckwith et al.[15] found that all fished had developed skin papillomas over time, but not an invasive skin cancers.

Transplantation of Mammalian Cancer Cells into Zebra fish

Different groups have been experimenting by transplanting mammalian cancer cells into zebra fish embryos. This leads to an in vivo system in which the advantages of cultured human cancer cells are combined with those of transparent zebra fish embryos in which development can be seen. Lee et al. [16] transplanted fluorescently labeled human metastatic melanoma cells into zebra fish blastula-stage embryos and showed that these cells survive, migrate, and divide, and are still present in adults but does not cause cancer or

metastases. one more study showed that aggressive human melanoma cells are able to induce a secondary axis or an abnormal head when transplanted into 3-hour-old zebra fish embryos, which was shown to be due to Nodal signaling from the tumor cells [17, 18]. In contrast to the above studies where no cancer development was observed, similar human melanoma cells as well as a colorectal and a pancreatic cancer cell line were found to induce tumor-like cell masses when transplanted into 2-dayold zebra fish embryos [19]

Zebra fish Cancers Compared with Mammalian Cancers

As earlier mentioned, zebra fish can develop any type of cancer [2]. Moreover, a marvelous asset of zebra fish as a cancer model is that many tumors histologically resemble human tumors. In addition, more general cancer characteristics such as genomic instability, invasiveness, transplantability, and the existence of cancer stem cells [40] apply to zebra fish tumors as well, and many tumor suppressor genes and oncogenes have been conserved. Taken together, these studies validate zebra fish as a bona fide cancer model. However, many details are still unknown and some important differences with regard to human tumor genesis have also become clear, which will be discussed below.

Carcinogen treatments on zebra fish give more vigorous induction of cancer and are considerably easier to perform as compared with the mouse. Even though early studies of transgenic fish expressing oncogenes yielded highly variable tumor incidences, it is exciting and promising to see that technical improvements boosted cancer incidences to 80% to 100% [36]. A recurrent problem in zebra fish is the presence of duplicated genes resulting from recent partial or complete genome duplication in teleosts [53], which may manipulate the role of oncogenes and tumor suppressors in carcinogenesis. An example is the presence of two forms of pten (Phosphatase and tensin homolog) in the zebra fish genome, which were found to be functionally redundant in development but not in oncogenesis: no loss of ptena was observed in tumors of ptenb homozygous mutants [28].

In addition, although loss of heterozygosity is expected to be the frequent mechanism in humans to explain the cancer predisposition in individuals with heterozygous tumor suppressor mutations [54], it was hardly ever observed in the comparable zebra fish mutants: not in apc [12], not in all mutant lines of the retroviral insertion screen [24], only once in the whole set of genomic instability mutants [25], twice in the separate mutant (interestingly, these particular mutants did not show a polyploidy phenotype [13], and never in the bmyb mutant [14]. An interesting study in this respect is the N-nitrosodimethylamine induction of liver tumors in triploid and diploid zebrafish [9]. When pretentious that loss of function of tumor suppressor genes is an important step in cancer development, one can expect that in case of a triploid fish, three alleles need to be hit to lose the function of the gene, consequently taking longer for

tumors to develop. Indeed, triploid fish had a slightly later onset of cancer, but concomitantly these fish showed a higher incidence of tumors, which indicates that activation of oncogenes, is equally important.

Tumor Spectrum

Most tumor classifications in zebra fish are still more accurate, lacking the more refined identification marker staining available for mammals. Unfortunately, many commonly used assays for human and mouse do not work on zebra fish material, despite repeated efforts from probably almost many zebra fish tumorigenesis labs. The Cheng lab is working towards a systematic zebra fish tumor histology database, which is a very valuable initiative to standardize tumor classification. Nevertheless, the need for zebra fish-specific antibodies is evident for this and many other research areas. The most common target tissues for spontaneous tumors in wild-type fish are testis and liver [2]. In contrast, genetic mutant lines most commonly develop MPNSTs, as observed in the p53, mismatch repair, ribosomal protein, and genomic instability mutants [24, 25, 27]. Likewise, the transgenic line over expressing human MYCN develops MPNSTs [43]. MPNSTs form a large group of tumors that includes, for example, neurofibromas, the tumor type that occurs in human neurofibromatosis conditions.

As already mentioned, defective mismatch repair in humans is linked to hereditary nonpolyposis colorectal cancer. However, this concerns patients, with heterozygous for the mismatch repair gene mutation. Interestingly, the rare patients with biallelic inactivation of mismatch repair genes have been reported to develop brain tumors. Mouse mismatch repair knockouts mainly develop lymphomas [48], indicating that the zebra fish models important aspects of the human disease that are not observed in mice. p53 mutants in human and mouse do not develop MPNSTs but rather osteosarcomas, breast cancer, brain tumors, and leukemias in human [49], and lymphomas and sarcomas in mouse .

The intestinal and liver tumors in zebra fish apc mutants [12] are comparable to those in mouse mutants and human patients, also with regard to constitutive activation of the Wnt signaling pathway in tumors [45, 50]. Because gut of the zebra fish is much smaller than that of mouse and human, the absolute chances for developing gut cancer are probably proportionally smaller, perhaps explaining the observed difference in penetrance. Shockingly, in zebra fish ptenb mutants, only one type of tumors was found inside the eye [28], whereas mouse and human pten mutants showed a broad spectrum of cancers [46, 51]. For the bmyb mutant, gene expression profiles of homozygous embryos showed significant correlation with human cancers [14], which is important, as a developmental zebra fish phenotype was compared with a human cancer.

A extensive expression array study was done by 7,12-dimethylbenz[a]anthracene – and 1,4-diphenyl1,3-

butadiene –induced liver tumors in zebra fish, showing significant similarities with human liver tumors, but not with other human tumor types [55-57].Two human types of rhabdomyosarcoma, embryonic rhabdomyosarcoma and alveolar rhabdomyosarcoma, have a distinctive molecular signature. Microarray analysis on rhabdomyosarcomas of activated RAS transgenic fish showed that these have a signature similar to human embryonic rhabdomyosarcoma but not to alveolar rhabdomyosarcoma. The profile also showed similarities to other RAS-induced tumors such as human pancreatic adenocarcinoma and mouse lung adenocarcinoma, in addition to a more specific embryonic rhabdomyosarcoma signature of muscle genes [40]. These studies collectively indicate that the genetic pathways involved in cancer development are conserved between fish and mammals.

Specific Advantages of Zebra fish

The difference of zebra fish cancer from mammalian cancers do not compromise the organism as a cancer model, but should rather help focusing on the specific strengths of zebra fish to disentangle mechanisms in carcinogenesis, corresponding to other models. Screens the suitability of zebra fish for genetic screens has long been recognized also in cancer research. However, although very elegant strategies were chosen for the first screens for cancer genes that were done in zebra fish, the results were promising but not completely convincing. Two new genes for which a relation to cancer was unknown or unclear were identified in the proliferation screen, but the cancer predisposition in heterozygous mutants is marginal and only visible when carcinogen treatment was used [13, 14]. In relation to this, no mutations in separate, one of the identified genes were found in 82 human tumor cell lines. In addition, although the ribosomal protein mutants from the retroviral insertion screen were clearly cancer-prone, a clear connection of this important and large group of genes to cancer had never been observed in other organisms [24]. Certainly, further research is necessary to qualify these genes as real ''cancer genes.'' The mutants from the genomic instability were all clearly predisposed to cancer [25], but because the embryonic phenotype is a matter of chance and not fully penetrate, mapping the mutants is labor intensive and time-consuming. However, there are many options for advanced novel screens by performing mutagenesis in a cancer-prone background or crossing mutants to a cancer prone line to identify genetic modifiers of specific cancers. Additionally, making use of transgenic lines with fluorescent reporters and/or chemical compound libraries will certainly result in new steps in cancer research. Imaging the lauded advantage of zebra fish embryos being transparent does not, except for some transplantation studies, apply to most cancer studies in zebra fish that involve adult animals.

However, a relatively transparent adult zebra fish line that lacks all types of pigment has been generated by Znomics [58]. Additionally, Goessling et al. [59] have successfully applied high-resolution microscopic ultrasound to follow tumor development and regression by treatment in living adult fish. Other existing imaging techniques that should, in principle, be possible in zebra fish are tomography and magnetic resonance imaging. [58]

Another example of the versatility of fluorescence in zebra fish is the generation of transgenic zebra fish lines expressing mammalian oncogenes, where the expressions are usually provided with fluorescence markers to visualize the carcinogenesis process refs. [33, 35, 39, 41, 43]. For example, Langenau et al. [38] used fluorescence of labeled leukemic cells to show that radiation treatment of wild-type fish transplanted with leukemia cells resulted in disappearance of cancer, whereas in bcl2-overexpressing, apoptosis-deficient fish the cancer remained present. Fluorescence was also very elegantly used for tracking recombination events in cre/loxregulated systems in whole fish. A loxed dsRED gene in between the rag2 promotor and EGFP-mMYC transgene resulted in dsRED expression in thymocytes, but on recombination in the presence of CRE, resulted in a switch to enhanced green fluorescent protein (EGFP) expression in the same cells [36, 37]. In the rhabdomyosarcoma model mentioned above, different fluorescent markers were successfully used for the fluorescence-activated cell sorting of four distinguishable cell types to identify cancer stem cells that have the capacity to induce new cancers in transplanted fish [40]. The above examples are pioneering studies, but they already show the enormous possibilities of using fluorescence in zebra fish cancer research, being analytical of a proportional number of opportunities for new studies. For example, some of the transgenic lines will be very well suited for genetic or chemical screens to identify modifiers of carcinogenesis, potentially in automated high-content screening setups.

Chemical Treatments in Search for Drugs As already mentioned, chemical treatment of both embryos and adult animals is relatively easy for zebra fish. In this respect, zebra fish can be a adaptable model in search of cancer therapeutics. Some proof of principle comes from the effective use of known angiogenesis inhibitors in transplantation-induced vascular remodeling. The neovascularization inside mammalian tumor grafts in zebra fish embryos could be inhibited by treatment with antiangiogenic drugs, whereas development of the normal vasculature in these embryos was not influenced [20]. One of those compounds was also found to block the angiogenic response to human tumor cell – secreted vascular endothelial growth factor in zebra fish embryos [21]. Another type of search for therapeutics concerns that of radio protective agents, which are of great clinical relevance considering the importance of radiotherapy in human cancer treatment. As an example, the nanoparticle DF-1 was shown to reduce ionizing radiation damage in zebra fish embryos [60]. The state of the art would be to screen chemical compound libraries to identify novel drugs that inhibit certain aspects of cancer

development. For this purpose, mutants that are embryonic lethal in the homozygous state and cancer-prone in the heterozygous state, such as those from the retroviral insertion and proliferation screens or the apc mutant [12-14, 24, 30], can be very useful because the early embryonic phenotype can be used as a readout whereas the obtained compound may very well be applicable to the adult cancer phenotype. In a nice and successful example of this, the bmyb mutant was used for a small-molecule screen in which the novel compound persynthamide was found to rescue its embryonic phenotype [61, 62]. Unfortunately, the effectiveness of this compound in adults has not yet been reported. Similarly nice small-molecule screen identified the compound 4-bromo3-nitropropiophenone as a radiation sensitizer specifically for cancer cells. In zebra fish embryos transplanted with human cancer cells and treated with this compound, tumor growth was inhibited by irradiation while there was no effect on embryonic development [62]

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