Zebrafish as a cancer model system

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The zebrafish, with its combination of forward genetics and vertebrate biology, has great potential as a cancer model system.

Cancer is an unfortunate reality for millions of humans worldwide, and a fact of life for all vertebrate organisms. Although invertebrates such as nematodes and flies can develop abnormalities in cell proliferation, what we recognize clinically and pathologically as cancer is present almost exclusively in vertebrates, from fish to humans. Thus, to understand the formation, growth, and spread of malignant tumors, vertebrate models are necessary.

We propose that the zebrafish is an ideal vertebrate system in which to model cancer. Despite the more than 300 million years separating the last common ancestor of fish and humans, the biology of cancer is very much the same in these two organisms. Cancer is commonly seen in fish in the wild, and straightforward assays involving water-borne carcinogen exposure have demonstrated that teleosts develop a wide variety of benign and malignant tumors in virtually all organs, with a histology closely resembling that of human tumors (Hawkins et al., 1985; Spitsbergen et al., 2000; Figure 1). As in humans, cancer in fish is a genetic disease, as shown by the melanomas that develop in *Xiphophorus* hybrids (Walter and Kazianis, 2001).

A comparison of the human genome sequence and the soon to be completed zebrafish sequence demonstrates conservation of cell-cycle genes, tumor suppressors, and oncogenes. Beyond comparative genomics, there are many advantages to modeling cancer in the zebrafish system (Patton and Zon, 2001; Figure 2). For example, large scale, forward genetic screens can be targeted to these highly conserved cancer pathways. Although cancer is primarily a disease of adults, mutagenesis screens could be designed to examine cell-cycle phenotypes in the transparent, rapidly developing embryos. Examples of such screens already underway include those focusing on cell proliferation (J.L.S., J.F.A., and L.I.Z., unpublished data) and cell differentiation and genomic instability (K. Cheng, personal communication). Genetic screens in yeast, *Drosophila*, and *C. elegans* have already revealed key genes regulating the cell cycle, cell proliferation, and apoptosis. Similar screens in the zebrafish would examine the conservation of gene function in these biological pathways, and establish any vertebrate-specific events that lead to cancer. Most importantly, via the carcinogenesis assay, the zebrafish system provides a direct way to test if a mutation causing an embryonic phenotype also causes a cancer predisposition in adults.

Once a cancer-related mutation is found, it is possible to identify interacting genes via suppressor/enhancer screens. In zebrafish, it is possible to perform mutagenesis-based modifier screens and directly address the role of the modifier in cancer, because a modifier of the embryonic phenotype may reduce the cancer predisposition in adults. Imagine using a zebrafish strain with a tumor suppressor gene mutation to find a second gene which, when inactivated, prevents cancer formation. This modifier becomes an excellent target for an antineoplastic drug.

In addition to traditional genetic screens, the zebrafish system is amenable to chemical genetic screens. Large numbers of embryos can be arrayed into multiwell plates containing water and aliquots of small molecules from chemical libraries (Peterson et al., 2000; H.M.S., R. Murphey, and L.I.Z., unpublished data). Chemical screens using embryos would select for drugs active in a multicellular organism, an advantage over tra-

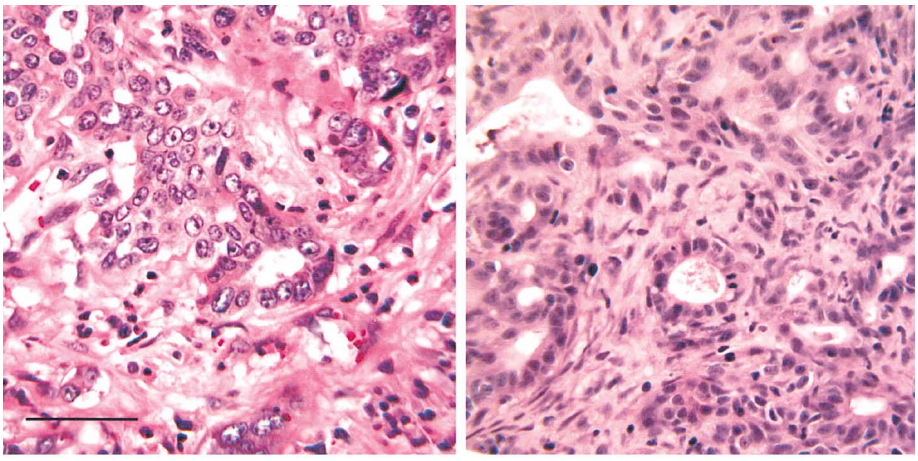

Figure 1. Histology of cholangiocarcinoma in human and zebrafish

Cholangiocarcinoma is a malignant bile duct neoplasm that occurs in both humans and zebrafish. The histologic appearance, including atypical nuclei, haphazard arrangement of irregularly shaped glands, and increased mitotic activity, is very similar in the two organisms. Bar is 50 μm.

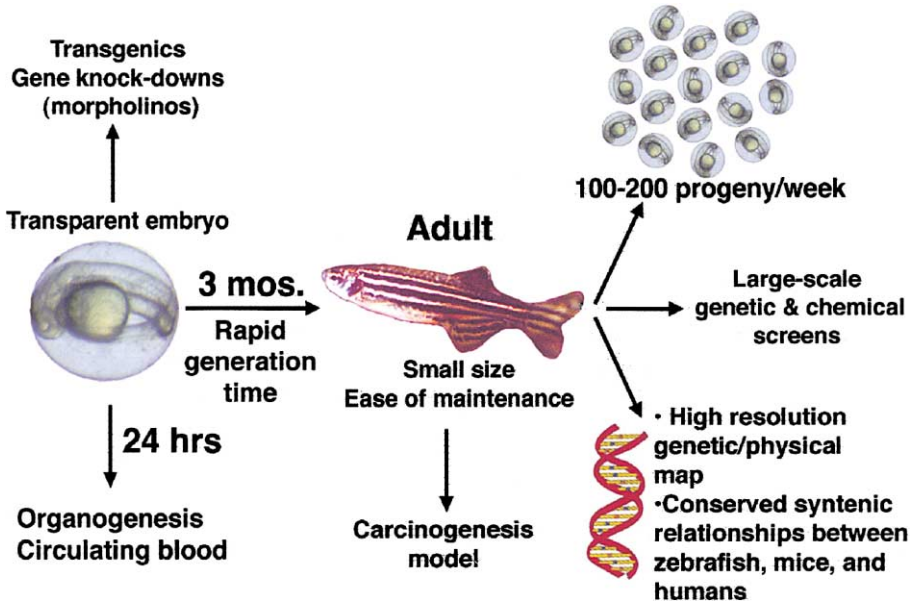

Figure 2. Strengths of the zebrafish system

The zebrafish is an ideal complement to existing genetic systems. Like flies and worms, the transparent embryos are produced in large numbers and are accessible for rapid screening and experimental manipulation. Like mice, zebrafish have vertebrate anatomy, physiology, and tumor biology.

ditional screens using transformed cell lines or in vitro protein binding assays. Embryos exhibit many features of cancer, including rapidly dividing cells, extensive apoptosis, and vigorous angiogenesis. Screens for compounds that affect these embryonic properties could identify compounds that are useful for the treatment of cancer or are tools to study cancer pathways. For instance, in this issue of *Cancer Cell*, Chan et al. (2002) demonstrate that a specific inhibitor of the vascular-endothelial growth factor, flk1, leads to defective angiogenesis. Such an angiogenesis inhibitor could be formally tested as an antineoplastic agent in a cancer-prone zebrafish mutant. A particularly exciting approach would be to combine the power of zebrafish genetics with chemical genetics by performing a chemical suppressor/enhancer screen on a zebrafish cancer model. One of the major challenges in the field will be development of high-throughput methods to screen compounds using embryos.

The mouse is an extremely important cancer model, based both on its similarity to humans and on the many tools that have been developed (Tuveson and Jacks, 2002). In order for the zebrafish to advance as a cancer model system, researchers will have to develop comparable technologies. Establishing growth of tumor cells both in cell culture and as xenografts or allografts would be invaluable. Transgenic lines could be used to express oncogenes, or to delineate gene expression through the use of green fluorescent protein (GFP). Tissue-specific, inducible, or recombinase-based transgenics are needed, similar to mouse models. While gene inactivation by homologous recombination remains to be developed, rapid analysis of gene function in zebrafish is possible using morpholinos (Nasevicius and Ekker, 2001). Morpholinos are antisense, chemically modified oligonucleotides typically directed against the 5′ region of the coding sequence of a gene and have been successfully used to phenocopy many mutants. This gene "knock down" approach is effective during embryogenesis, but the morpholinos degrade after about 4 days. Thus, the morpholino technique does not permit assessment of the long-term effects of gene inactivation. Nevertheless, this 4 day time period is adequate to evaluate cellular processes related to cancer biology and determine if the pathways found in mammals are present in the fish.

To facilitate the use of zebrafish as a forward genetic tool, the speed and efficiency of mutant screening and gene cloning needs to be improved. For example, while ethylnitrosourea (ENU) mutagenesis is relatively efficient, recovery of mutations is still time-consuming. The Sanger Center sequence of the zebrafish genome, taken together with trans-NIH efforts in both genetics and genomics, will greatly help. A different approach is the use of retroviral insertions (Amsterdam et al., 1999) or transposons, which facilitates cloning of the mutant gene. The power of any screen to detect mutations depends critically on the tools used to detect the phenotype. At this point, relatively few commercial antibodies exist that recognize zebrafish cell cycle, signaling, and apoptosis-related proteins. One alternative may be the use of transgenic lines; GFP reporters that mark specific aspects of cell proliferation and survival could be used both for genetic screens and high-throughput small molecule screens.

The zebrafish cancer system can be viewed as a combination of vertebrate tumor biology, classical and chemical genetics, and genomics. Beginning with the pioneering work of Streisinger and colleagues, the zebrafish was envisioned as an excellent model system for complex biology. The large-scale forward genetic screens in Tübingen and Boston in the 1990s furthered the system in order to understand early embryonic development (Driever et al., 1996; Haffter et al., 1996). Many of the mutants obtained in these screens represent animal models of rare genetic diseases. Now, we propose that the zebrafish be used to attack a common disease, cancer. The magnitude of the challenges facing zebrafish cancer research is matched by the great promise of the system to discover novel cancer genes, to probe the interactions among these genes, and to identify chemotherapeutic and chemopreventive agents in the context of a living, vertebrate organism.

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