The Zebrafish & Disease Project: Zebrafish as a model system to study and cure human diseases

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During the past 20 years zebrafish has served as an excellent model for understanding normal development and birth defects based on its powerful genetics and exquisite embryology. More recently, research with zebrafish has extended to model human diseases and to analyze the formation and functions of cell populations within organs. This work has generated new human disease models and has begun to identify potential therapeutics, including genes that modify disease states and chemicals that rescue organs from disease. For example, recent breakthroughs made in zebrafish include the isolation of a human skin color gene, the development of a melanoma model, and the isolation of a chemical that can correct cardio-vascular defects. Building on the exceptional intellectual and collegial openness of the zebrafish research community, a tremendous number of new investigators with disease-specific interests have been welcomed and rapidly trained in the field.

A recent meeting of the zebrafish community's primary investigators (September 14-17, 2005) led to a plan to create a discovery engine that will change the way we understand human diseases and treat them. This large-scale project will create mutations in zebrafish orthologs of human disease genes, generate transgenic animals for phenotypic analysis and disease modeling, and use chemical genetics to find therapeutic agents. Importantly, this project is not feasible or cost-effective in any other vertebrate model system. This plan will create a platform to study and cure human diseases and also benefit other research areas, from stem cells to neural circuitry. Below is a plan describing the community's objectives.

1. Reverse genetics: Generate mutations in zebrafish orthologs of human disease genes.

A number of strategies in the zebrafish system rely on forward genetics. This entails making a mutation and then finding the gene. Over the past three years, techniques to derive mutant zebrafish using reverse genetic strategies have also proven beneficial. For instance, the process of targeted lesion detection (or TILLING) has uncovered mutant models of human diseases. This strategy has identified p53 mutant zebrafish that have a predisposition to cancer and rag1 deficient zebrafish with immunodeficiency. Creating mutant zebrafish lines by TILLING, retroviruses or transposable elements dramatically simplifies disease modeling because of the powerful cellular and genetic approaches available in zebrafish to monitor disease progression. Moreover, suppressor and enhancer screens on these disease models, both using forward genetics and chemicals, provide excellent ways of modifying the disease phenotype. Such studies can identify genes that interact to produce models of multigenic human diseases and to identify drugs to treat them. A major advantage of the planned work in zebrafish will be the ability to generate a large range of gene changes that reflect the spectrum of alleles that predispose to disease in humans or only inactivate a gene product at a particular temperature. The zebrafish research community seeks funding to create a knock-out and allelic series for 2000 genes linked to human disease and key signaling pathways. Mutant zebrafish lines will be generated by a consortium of centers and distributed by a stock center. Data will be accessible on the zebrafish internet based information network, ZFIN (http://zfin.org), and NCBI. This project will provide a unique resource for both current zebrafish researchers and scientists in other fields.

2. Transgenesis: Generate tools for inducing and modeling human diseases in vivo.

The zebrafish offers the unique opportunity to follow normal and aberrant developmental and

physiological processes in vivo at subcellular resolution. In particular, transgenic animals can be continuously monitored and confocal imaging can be used to study behaviors of cell populations highlighted by fluorescent proteins in vivo and in real time. Recent improvements in transgenesis methods have made the zebrafish a dramatically more efficient and cost-effective system than the mouse. In addition, transgenic zebrafish have been used to create human disease models. This is particularly important, because many human diseases are caused by the ectopic expression of genes. For example, a transgenic zebrafish carrying the activated BRAF gene implicated in human skin tumors leads to large melanomas. The zebrafish research community proposes to develop several thousand zebrafish strains that mark every tissue and cell type of the body and that allow the misexpression of human disease genes. This can be achieved by generating transgenic lines in which a reporter or disease gene is expressed under the control of a tissue- or cell type-specific promoter. To generate a system that allows the tissue-specific expression of any protein of choice, several thousand transgenic lines will be generated that express an inducible transcription factor or recombinase in specific tissues and cells and that can activate the expression of any gene of interest. These transgenic zebrafish lines will be generated by a consortium of laboratories and distributed by a stock center. Data will be accessible via ZFIN (http://zfin.org) and NCBI. This transgenesis project will provide in vivo markers for mutant screens and the study of disease progression at cellular and subcellular resolution, and generate models for human diseases caused by gene misexpression.

3. Chemical genetics: Find drugs that suppress diseases.

The recent development of the zebrafish as a model for chemical genetics has established chemical screening in vivo as an adjunct to older screening technologies in cell lines or in vitro. Soluble chemicals permeate into zebrafish embryos and produce specific effects. For instance, 50 – 70% of the chemicals known to cause abnormalities of the cell cycle in mammalian cell lines also affect the zebrafish cell cycle *in vivo*, and drugs blocking prostaglandin synthesis are as effective in zebrafish as in humans. In contrast to screening by *in vitro* techniques, zebrafish offers an *in vivo* vertebrate model for studying the bioactivity of chemicals. In addition, the availability of large numbers of zebrafish mutants makes chemical suppressor screens fast and straightforward. For example, one screen identified a chemical that suppressed a specific cardiovascular disease. The zebrafish research community proposes to assign two centers to (1) distribute chemical libraries to individual labs in a format for zebrafish experiments and (2) perform large-scale screens for chemicals that suppress genetically caused diseases. The centers will provide fish holding and screening/imaging facilities for zebrafish researchers and could undertake more than 24 large scale screens with up to 100,000 compounds and over 200 individual screens with about 2000 bioactive compounds. Data will be collected by ZFIN (http://zfin.org) and NCBI. The targets of chemicals found to prevent or cure disease phenotypes in zebrafish will, in general, have very close cognates in humans. Therefore these screens promise to provide key entry points for the development of new therapeutic drugs.

Summary

In summary, the zebrafish provides a unique advantage for large-scale vertebrate genetics combined with exceptional visualization of cell populations and physiological processes. The ability to make models of human diseases coupled with suppressor and enhancer screens and chemical screens will make the zebrafish an even more important contributor to our understanding of human disease. The proposed projects will complement the ongoing NIH initiative that supports inventive screens and tool development (PAR-05-080). The zebrafish primary investigators agree that the combination of reverse genetics, forward genetics and chemical genetics will provide fundamental insights into human diseases and their cure.