Zebrfish With Antisense-Knockdown of Cardiac Troponin C as a Model of Hereditary Dilated Cardiomyopathy

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Functional abnormalities of cardiac troponin caused by either mutations or auto-antibodies can induce dilated cardiomyopathy (DCM),1,2 which is characterized by left ventricular enlargement and systolic dysfunction resulting from a reduction in the myocardial force of contraction. DCM causes congestive heart failure, which can be treated with diuretics, angiotensin-converting enzyme inhibitors (or angiotensin type-I receptor blockers), β-adrenergic blockers, and most aggressively in unresponsive patients with cardiac transplantation. However, the prognosis of DCM is still poor, so there is a need for new therapeutic approaches.

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Approximately 20–50% of cases of idiopathic DCM may have a genetic basis. Screening first-degree relatives of a proband with idiopathic DCM by echocardiography and electrocardiography reveals that 20–48% of probands have affected relatives, consistent with a diagnosis of familial DCM. Numerous large kindreds with familial DCM have provided the foundation for establishing genetic causation, and mutations in multiple genes have been shown to cause as shown in the website of GENE Reviews.3 Current estimates indicate that the 20-plus known familial DCM-causing genes account for only a minority of cases of this disease. To understand its pathology, and with the ultimate hope of finding a treatment, researchers have generated genetic rodent models of heart failure.4,5 On page 1691 of this issue of the Journal, Ho et al6 describe their attempt to establish a transgenic model of idiopathic DCM in zebrfish using microinjection of tissue-specific and inducible RNA interference with cardiac troponin C (TnC).

Myocardial contraction results from interaction of the myosin with the actin in the myocardial thin filament. The thin filament is comprised of actin, tropomyosin and troponin. Troponin is a complex of 3 subunits, troponin T (TnT), troponin I (TnI), and TnC, each of which has distinct roles in the thin filament. Elevated [Ca²⁺] leads to a conformational change in TnC, increasing its affinity for TnI, while the affinity of TnI for actin decreases. The conformational change in TnC, increasing its affinity for TnI, while in the thin filament. Elevated [Ca²⁺] leads to a conformational change in TnC, increasing its affinity for TnI, while in the thin filament.

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to hemodynamic collapse or conduction abnormalities similar to that observed in human patients with endstage heart failure.

Drug-controllable antisense-knockdown allows for conditional expression of endogenous genes, an objective of great value in many areas of basic and applied research. However, it typically requires extensive empirical optimization of the target region along the mRNA molecule, which becomes an insurmountable obstacle when the target gene belongs to a family of closely related members with partial homologies throughout their coding sequences. Furthermore, antisense oligonucleotides can lead to unexplained effects when introduced into the cell. In this study, it is surprising that the antisense-knockdown consequently inhibited the translation of cardiac TnC, even though endogenous mRNA of cardiac TnC was detected in the cardiac tissue of the transgenic zebrafish. On the other hand, RNA polymerase II promoters, including the tet-responsive promoter, can be used to express short hairpin RNAs based on microRNA precursors. The transgenic RNA interference technique eventually might be used to spatially, temporally and reversibly regulate the expression of any endogenous gene. These methods hold promise as an experimental tool for loss of function genetics.

The zebrafish has gained popularity as a useful animal model in scientific research because its embryo develops rapidly and preserves the fundamental body structure, which is similar to that of humans. The heart beat is observable at 1 dpf and on day 4 it can swim and feed. Because of its transparency, organ development and function in zebrafish embryos can be observed at these stages. Transgenic zebrafish expressing fluorescent protein under the regulation of tissue-specific promoters makes it possible to visualize certain organs at defined developmental stages. Drug-induced gene expression by fluorescent protein as a reporter can also be evaluated. Zebrafish produce hundreds of eggs in 1 week, enabling the rapid preparation of a large number of individuals with the same genotype. Furthermore, experimental procedures using microformats, such as microplate and automated large-scale analysis, are facilitated.

Recently, research using zebrafish is expanding into pharmacology and clinical research as a model and in drug discovery. The use of zebrafish in pharmaceutical research, discovery and drug development can be used for screening of lead compounds, target identification, target validation, morpholino oligonucleotide screens, assay development for drug discovery, physiology-based drug discovery, quantitative structure–activity relationship, and structure–activity relationship studies, and drug toxicity studies. Morpholino oligonucleotides can knockdown gene expression, modify splicing or inhibit mRNA activity and maturation. It is one of the best antisense reagents for cells in culture and the embryos of zebrafish are an indispensable tool of developmental biology. The use of caged morpholino antisense oligonucleotides, activated by light, enables temporal control of gene knockdown. However, this method not only has transient effects but also off-target effects. Targeted knockout approaches have been very successful in mice, but are currently not feasible in zebrafish because of the inability to grow embryonic stem cells. Instead, Ho et al. established proof of the principle of a transgenic antisense technology that might be eventually be used to spatially, temporally and reversibly regulate any endogenous gene. As another alternative, a reverse genetic approach that uses screening by resequencing and/or Targeting Induced Local Lesions IN Genomes (TILLING), which enables detection of rare point mutations in genes of interest in chemically mutagenized genomes, has recently gained popularity in the zebrafish field. Zebrafish may be a promising model in biomedical research, development research and drug discovery in forthcoming years.

In addition, Wolf et al. recently indicated that transgenic Drosophila with a mutation in TnI or tropomyosin, and with inducible cardiac expression of a mutant of human δ-sarcoglycan (δsgk1A), which has previously been reported as associated with familial cardiomyopathy, developed marked impairment of systolic function and significantly enlarged cardiac chambers. Of course, Drosophila are not transparent, so the researchers used optical coherence tomography to observe the cardiac movement. Drosophila also may be a promising model for systematic study of the genetic mechanisms responsible for human DCM. Thus, the work of Ho et al. is an important advance in developing a transgenic zebrafish model of DCM that should allow further advances in the understanding and treatment of this important condition.

References


15. Till BJ, Zerr T, Comai L, Henikoff S. A protocol for TILLING and Targeting Induced Local Lesions IN Genomes (TILLING), which enables detection of rare point mutations in genes of interest in chemically mutagenized genomes, has recently gained popularity in the zebrafish field. Zebrafish may be a promising model in biomedical research, development research and drug discovery in forthcoming years.