Advances in Medical Ethics

Zebrafish *in vivo* Testing of microRNA-mediated Gene Knockdown

Khaled Sayeed*

Department of Pharmaceutical Technology, American University of Afghanistan, Near Palace of Darual Aman, Darulaman Rd, Kabul, Afghanistan

DESCRIPTION

Due to the abundance of orthologous genes that encode proteins that are comparable to those found in humans, the zebrafish (*Danio rerio*) has emerged as a promising model for the study of human disease. The zebrafish genome can only be modified with a small set of tools, and many of the methods now in use are either successful in the early stages of development (such as morpholino-based antisense technology) or are phenotypically driven without providing specific gene knockdown (such as chemical mutagenesis). The use of RNA interference has been controversial because off-target effects can make it challenging to interpret phenotypic results. This problem has been handled by developing zebrafish lines with miRNA constructs that are stably integrated and target the desired gene of interest. In this study, we demonstrate that a commercially in a temporary *in vivo* eGFP sensor test setup, the miRNA backbone is successful in producing eGFP knockdown in zebrafish. We decided to use this technique to knockdown transcripts associated with Long QT syndrome, a human heart disease.

Since the zebrafish genome contains many orthologous genes and the encoded proteins have similar roles to those produced in humans, the zebrafish has emerged as a promising model for the simulation of human diseases. There are just a few techniques for changing zebrafish gene expression, but the most effective one is morpholino-based antisense technology, which enables transitory downregulation for the purpose of researching the impacts of decreased gene expression during early vertebrate development. Chemical mutagenesis (ENU) is the preferred technique for producing stable mutant fish lines, although this forward genetics strategy is based on phenotypic outcomes and is unable to target and knock down specific genes.

Zinc Finger Nucleases (ZFNs), on the other hand, are recently developed technique that have been successful in targeted gene knockdown in zebrafish. Unfortunately, the production and design process is laborious and necessitates specialized knowledge, which limits its utilization in the larger academic community. Additionally, ZFNs do not provide the option of an conditional gene knockdown or tissue-specific gene knockdown (the ability to turn on or off gene knockdown). The latter result would offer a more nuanced disease model appropriate for researching late-onset illnesses, and in this situation, RNA interference would be a promising new approach.

In mammalian systems, the application of RNA interference (RNAi) technology has proven to be a highly helpful tool for disease modeling. Since different promoters can be employed, gene silencing can now be restricted to specific tissues or organs while also enabling conditional silencing using Tet-on promoters (the Cre/loxP system is used in mice). In short, tiny noncoding RNAs allow for posttranscriptional fine-tuning of gene expression throughout development, apoptosis, and metabolism. These non-coding RNAs can be synthesized to knock down genes and cause the cleavage/degradation, or the halting of translation, of a target mRNA. Using a DNA-based vector system, miRNAs can be expressed. miRNA transcripts, which have a double-stranded hairpin structure, are broken down by multiple endonucleases to create 22 miRNAs that have reached their nucleotide maturity subsequently join forces with the RNA-Induced Silencing Complex (RISC) to silence specific genes. As there are differing views on the efficiency and specificity of RNAi in producing gene knockdown, its application in zebrafish has been contested. MiRNA expression in zebrafish embryos has been theorized to cause toxic/off-target traits by overwhelming the endogenous miRNA pathway. But it has been possible to create transgenic zebrafish lines that express miRNAs with no discernible harmful effects that target desired genes of interest. MiRNAs were effectively exploited by Dong et al. and Ho et al. to mediate gene knockdown in zebrafish, and they also developed conditional knockdown models and heritable gene knockdown fish lines.

In light of the foregoing context, we have thought about how RNAi-based technology can enable the creation of zebrafish models of heritable cardiac illnesses like Long QT Syndrome (LQTS). A prolonged QT-interval, which can cause deadly arrhythmias, characterizes this syndrome, a congenital heart ailment. We have concentrated on the *KCNH2* gene, which codes

Correspondence to: Khaled Sayeed, Department of Pharmaceutical Technology, American University of Afghanistan, Near Palace of Darual Aman, Darulaman Rd, Kabul, Afghanistan, E-mail: sayeedk152@gmail.com

for the subunit of the rapid delayed inward rectifier potassium channel and is essential for the repolarization phase of the cardiac cycle, among the other genes (encoding for cardiac ion channels/scaffolding proteins) implicated with LQTS. Among the dominant mutations found in LQTS, type 2 patients are nonsense mutations, exon deletions, and duplications, which show that *KCNH2* haploinsufficiency contributes to the symptoms of disease.

Zebrafish contain two KCNH2-type genes that have been named zerg-2 and zerg-3. While the latter appears to encode a protein with *KCNH2* function but is an orthologue of the human *KCNH6* gene, another potassium channel that is in the same family as *KCNH2*, the former appears to be an orthologue of the human *KCNH2* gene.

CONCLUSION

Importantly, the (unregulated) suppression of both zebrafish genes results in a cardiac phenotype that resembles the phenotype experienced by people with LQTS (bradycardia, atrioventricular block, and prolonged QT interval). Here, we demonstrate that gene knockdown in zebrafish can be mediated *via* a miRNA vector technology that is commercially accessible.

We test this strategy using a temporary *in vivo* technique that specifically targets different areas of the zebrafish zerg-2 and zerg-3 genes transcripts as an initial step to imitate the haploinsufficiency of zerg-2 and zerg-3 in zebrafish in a stable transgenic fish line and to confirm that the specifically designed miRNAs may cause the knockdown of their intended targets.