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Gene-edited zebrafish models take disease research to the next level

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Thousands of zebrafish are organized in Seattle Children's Research Institute's aquatics facility based on the different mutations they carry in genes being studied. Credit: Seattle Children's

Advances in optimisation of the gene-editing technique CRISPR/Cas9 in zebrafish disease models offer a new level of accuracy and specificity previously out of reach for research into human genetic disorders.

A wide variety of diseases that affect people of all ages across the globe, including dementia, developmental disorders, some forms of cancer, muscular dystrophies and heart conditions, result from mutations in our

genes. As scientists seek to understand the genetic basis of these diseases and to work towards the development of effective treatments, having genetic models of the diseases is key to their research. While much of research today is carried out in cell lines, scientists also need to understand how a gene causes pathogenicity at the whole organism level. Animal models of disease allow researchers to test the functions of genes that may depend on interactions between different cell types within the animal, or that might affect the healthy development of embryos as they grow.

Experimental power in a small package

While mice or fruit flies will usually come to mind when thinking of animal models in the lab, zebrafish are increasingly popular for a number of important reasons. Due to their small size and inexpensive husbandry, they can be housed in large numbers. They have a relatively short life cycle, reaching sexual maturity in 2-3 months and producing up to 300 eggs per week. The embryos develop externally and are transparent, making them an ideal tool for non-invasive microscopic investigation as they develop. Zebrafish embryos are also an excellent tool for drug screening, thanks to their availability in large numbers and substantial contact surface with the water in which they grow.

In order to develop animal models of disease, scientists need to be able to manipulate the genes of animals to replicate the genetic defect that causes the disease. In the past, methods relied on knockdown of the entire gene or overexpression of modified genes harbouring patient-derived mutations, but these incompletely modelled the human disease in terms of protein expression levels or cell-type specificity, and also took a great deal of time to develop, even in zebrafish.

Gene editing opens new doors

CRISPR/Cas9 is the gene-editing technique that is currently revolutionising molecular biology, including disease model development. The system is derived from the bacterial immune response, where a short sequence from the DNA of invaders such as viruses is stored in the bacterial genome. If the bacterium is infected again, the stored viral DNA sequence helps guide a protein, Cas9, to the matching region in the viral genome, where Cas9 causes DNA breaks that lead to eradication of the invader. Scientists realised that the CRISPR/Cas9 system could be engineered to deliver Cas9 into cells together with a guide molecule to direct the exact site that DNA is cut. The immediate availability of freshly-fertilised zebrafish embryos makes them ideally suited for CRISPR/Cas9 gene editing, since embryos can be microinjected with reagents at the 1-cell stage, and as the embryo grows, new cells arising from this first cell will carry the target mutation.

Advancing 'knock-in' disease modelling

Crucially for disease modelling, when combined with a single-stranded template for DNA repair following the cut, CRISPR/Cas9 has enabled researchers to introduce precise point mutations to replicate those that cause disease in some human patients. However, while blanket 'knockout' of a gene with CRISPR/Cas9 is now relatively straightforward, introducing specific point mutations in a 'knock-in' model can still be problematic, particularly in zebrafish, requiring the screening of many animals to find individuals harbouring the desired nucleotide change. Three new articles published in *Disease Models & Mechanisms* explore the potential for using CRISPR/Cas9 to develop knock-in zebrafish models of human disease, and suggest new ways to overcome the issues still faced when creating models of human diseases caused by specific point mutations.

"There are almost no limitations on what we can design in zebrafish or other systems to generate models of human genetic disorders.

CRISPR/Cas9 has really for the first time made it feasible to test the effects of human disease-associated genetic variants in animal models," says Dr. Lisa Maves, lead author on an article from the Seattle Children's Research Institute and the University of Washington. Human genome sequencing studies often identify variants in gene sequences, even if the significance of these is not immediately clear. Maves' research team focused their work on using CRISPR/Cas9 zebrafish models to test whether gene variants identified in individuals with congenital heart defects contribute to development of the disease. "Vastly improving the efficiency at which specific nucleotide changes can be made to the zebrafish genomic sequence would be an important advance in the field," says Hank Farr, a member of the Maves Lab and first author on the article.

Also working to improve the current limitations to knock-in model creation, lead researcher Dr. Andy Willaert and his group at Ghent University have been investigating how point mutations in zebrafish with CRISPR/Cas9 and single-stranded repair templates can introduce errors into the zebrafish genome. "Zebrafish and CRISPR/Cas9 form the ideal duo for massive and swift disease model generation," says Willaert. "However, genome editing using a single-stranded repair template often occurs erroneously and commonly used analysis techniques do not always detect such erroneous repair. This can lead to a model with a gene disruption instead of knock-in and a possible misinterpretation of the experimental results obtained. As these models might be eventually used in therapeutic applications, we want to stress the possible occurrence of erroneous repair."

In The Netherlands, a team led by Dr. Jeroen Bakkers from the Hubrecht Institute and Dr. Gijs van Haaften from the University Medical Center Utrecht are researching the potential of CRISPR/Cas9 technology to develop patient-specific alleles for modelling human disease. The scientists used CRISPR/Cas9 and a short repair template to

generate knock-in zebrafish models of four human cardiovascular disorders caused by point mutations. Dr. Federico Tessadori, first author on the article, emphasises that, "the ability to introduce point mutations exactly replicating the situation in human patients is paramount for proper understanding of disease and for successful development of therapeutic strategies."

Despite the differences in approach and focus, the common message running through all three papers is that zebrafish have enormous potential for creating precise knock-in models of human disease caused by point mutations. However, all the authors emphasise the importance of screening for highly effective guide RNAs and single-stranded repair templates, and thorough testing for errors once the process is complete. Also important is the recognition that optimal experimental conditions often differ between disease model systems, and certain optimisation steps will always be necessary when using CRISPR/Cas9 to develop knock-in models in zebrafish or other model organisms.

Working towards better treatment for patients

Looking to the future, scientists will continue to improve CRISPR/Cas9 techniques to develop error-free knock-in zebrafish disease models. The use of zebrafish models of patient-specific mutations opens new opportunities for chemical screens and the development of new drugs. Ultimately, collaborations between researchers and clinicians caring for patients will bring even greater understanding of human genetic disorders that will help a family better understand their child's condition or guide a physician towards better treatments.

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