MOLECULAR BIOLOGY

Speeding up RNAi

A recent study turns the creation of conditional short hairpin RNA transgenic mice into a rapid, flexible and scalable process.

Manipulating gene expression in the mouse, either by overexpressing exogenous genes or by modifying endogenous ones, has for long enabled studies related to the function of these genes and their involvement in diseases. Ever since the discovery of RNA interference (RNAi), researchers have developed ways of exploiting this endogenous cellular mechanism of gene regulation to their advantage. In cells, the RNAi machinery recognizes and processes dsRNAs into small RNAs that guide the repression of complementary genes. If one introduces into a cell an exogenous dsRNA targeting a gene of interest, the expression of this gene can be repressed.

Despite its proven value, the production of RNAi transgenic mice for the study of gene function *in vivo* has not yet seen wide uptake. Scott Lowe and his collaborators at Cold Spring Harbor Laboratory think this is primarily due to the high variability of knockdown success among different RNAi transgenic mice and the inefficiency of the process involved in generating the mice in the first place.

Researchers in Lowe's laboratory have had a long-standing collaboration with RNAi expert and laboratory neighbor Greg Hannon. Among the joint projects they pursue is the development and application of RNAi technology to study how cancers develop. To knock down genes such as tumor suppressors and study the effect in the progression of cancer, the team uses dsRNAs called short hairpin RNAs (shRNA) for RNAi. One of the big advantages of using shRNAs for these studies is that the loss-of-function of the gene can be engineered to be reversible. "In the context of *in vivo* systems, we can use this to ask what the consequences of transient gene inhibition would have on a disease, and hopefully this will help validate drug targets more efficiently," explains Lowe.

But for this, the team first needed to optimize the production of shRNA transgenic mice. In their platform, any shRNA of choice is easily introduced into a genetic cassette in which shRNA expression is inducible and is linked to the production of a fluorescent reporter that allows tracking shRNA expression. Then, they introduce this construct into embryonic stem cells (ESCs) and target it to a single genomic locus using site-specific recombinase-mediated targeting. Finally, they use these cells to produce transgenic mice by tetraploid complementation or blastocyst injection.

Using this strategy, the authors generated eight tetracycline-regulated shRNA transgenic lines including several targeting tumor suppressors. They demonstrated potent and reversible gene silencing in a broad range of tissues *in vivo* and used the system to study the role that these tumor suppressors have both during mouse development and in cancer progression. Although site-specific integration of the transgene reduces variability, the authors still found some cells and tissues in which the expression of the shRNA was not as high as desired. They also found some 'leaky' expression of the shRNA in the absence of induction. These problems can be solved to a great extent using different promoters.

Lowe's group is particularly excited about applying this strategy to study the role of specific genes in the context of genetically engineered models of human disease. For this, they adapted the system to express any shRNA in the background of ESCs previously engineered to serve as disease models. The mice produced with this method, called 'speedy mice', enable researchers to rapidly determine the impact of gene suppression on the progression or elimination of the disease. The investigators examined the impact of the ARF tumor suppressor on lung cancer progression, but this strategy can be similarly applied to any genetic disease and easily scaled to examine multiple genes.

Understanding the biology behind complex diseases is a question that greatly interests Lowe. "We've learned so much from genomics and a lot of hypotheses have been generated but if you have to make six allele crosses to test one, you're never going to test ten," he says. "So I think this [pipeline] really allows us to test a lot more questions and quickly narrow in on the most relevant genes."

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RESEARCH PAPERS

Premsrirut, P.K. *et al.* A rapid and scalable system for studying gene function in mice using conditional RNA interference *Cell* **145**, 145–158 (2011).