MILESTONE 10

pSUPER start to large-scale RNA interference screening

Credit: Cultura Creative (RF) / Alamy Stock Photo

In 2002, Reuven Agami, Thijn Brummelkamp and René Bernards published two papers (in *Science* and in *Cancer Cell*) describing different versions of a plasmid-based expression system capable of driving continuous synthesis of a small interfering RNA (siRNA) in mammalian cells. These vector systems — pSUPER (suppression of endogenous RNA) and the retroviral version, pRETRO-SUPER — enabled targeted knockdown of gene expression with an efficiency matching that of synthetic siRNAs, yet without the loss of inhibition caused by prompt clearance of such siRNAs from mammalian cells, a disadvantage that had curtailed the applications of synthetic siRNAs.

Use of the pSUPER vectors achieved ~90% inhibition of the expression of the target gene and, in the case of pRETRO-SUPER, which integrates into the host genome, also enabled stable expression of the siRNA. These advances enabled the transfected cells to be cultured and the downstream effects of gene knockdown to be studied in detail.

Importantly, Brummelkamp et al. showed that RNA interference (RNAi) was highly sequence-specific: use of pRETRO-SUPER to deliver a *KRAS*-targeted siRNA inhibited the expression of mutant (oncogenic) *KRAS* mRNA without reducing the expression of the native mRNA. This specificity is essential for studying cancer, in which oncogenic and non-oncogenic alleles of the same gene (which might differ from each other by only a single nucleotide) are often co-expressed.

The pSUPER plasmid includes the promoter of H1 RNA polymerase III, placed upstream of a 19-nucleotide sequence derived from any gene of interest. This sequence is separated from its reverse complement 19-nucleotide sequence by a 9-nucleotide spacer, and is followed by a transcription termination sequence. Consequently, the RNA transcripts generated by pSUPER and pRETRO-SUPER self-fold into a stem–loop structure resembling a hairpin, and were therefore called short hairpin RNAs (shRNAs).

In their first 2002 paper, Brummelkamp et al. concluded that it should be possible to generate large collections of pSUPER shRNA vectors to carry out high-throughput genetic screens for loss-of-function phenotypes. Indeed, only two years later, the Bernards group demonstrated the utility of this system for large-scale screening. Berns et al. constructed a set of retroviral vectors encoding 23,742 distinct shRNAs, which targeted 7,914 human genes (that is, three different shRNAs targeting each gene) implicated in the promotion or suppression of cancer. The researchers then used this RNAi library to screen human cells and identified one known and five previously unknown components of the p53 tumour suppressor pathway, which induces cell cycle arrest, cell senescence or apoptosis. This study was the first to use vector-based shRNA libraries for large-scale functional genetic screening in human cells.

> “These vector systems… enabled targeted knockdown of gene expression with an efficiency matching that of synthetic siRNAs, yet without the loss of inhibition caused by prompt clearance of such siRNAs”

Other scientists were quick to realize the significance of this work. Agami, Brummelkamp and Bernards received thousands of requests for reagents from fellow researchers, and the use of siRNA-encoding (viral) vectors has since become a major method of gene suppression in mammalian cells.

Caroline Barranco, Senior Editor, Nature Reviews Cross-Journal Team

MILESTONE STUDIES Brummelkamp, T. R. et al. A system for stable expression of short interfering RNAs in mammalian cells. *Science* **296**, 550–553 (2002) | Brummelkamp, T. R. et al. Stable expression of tumorigenicity by virus-mediated RNA interference. *Cancer Cell* **2**, 243–247 (2002) | Berns, K. et al. A large-scale RNAi screen in human cells identifies new components of the p53 pathway. *Nature* **428**, 431–437 (2004).