How to kill your RNA

Switching off problematic genes with RNA interference promises treatments for a huge range of disease – if investigators can get it to where it’s needed. Lisa Melton reports
It all started with a petunia. Now, one Nobel prize later, this technique could prompt molecular medicine’s long-awaited revolution. The technology is RNA interference, and enthusiasts predict it could soon be used to treat every ailment—from cancer and pandemic flu to type 2 diabetes and heart disease—by shutting down rogue genes.

RNAi has gone from discovery into clinical trials with astonishing speed. Large pharmaceutical companies are signing billion-dollar deals to access gene silencing know-how—hedging their bets on its clinical potential. The stakes are high, but the rewards could be colossal.

There is a catch, however, and that is delivery. ‘RNAi reagents don’t necessarily go where you want them to,’ says Dmitry Samarsky, vice president of technology development at RXi Pharmaceuticals in Worcester, Massachusetts in the US. He presented his company’s latest research and spoke to Chemistry World at the RNAi Europe conference in Barcelona, Spain on 21 September 2007.

Getting RNAi therapies into specific parts of the body and across the cell membrane is the main challenge. ‘The great promise of selective gene silencing has been tempered by many barriers. The major barrier to using RNAi as a therapy is to move it from blood to inside the cell,’ says Paul White from Monash University in Australia.

Given that the hurdle is considerable, is the hype over RNAi therapeutics warranted? ‘People in the pharmaceutical industry are saying “the delivery issue needs to be resolved”,’ Samarsky said in his presentation. ‘Pharma always looks at it from the small molecule perspective: you gulp down an aspirin, it floods the body, it goes everywhere and does the job. We should be looking at RNAi in a different way.’

Fortunes are likely to be made from whoever resolves these issues, so no one is about to give up on medical applications. In fact, quite the contrary. The first generation therapies for age-related blindness, cancer, and respiratory syncytial virus are already being cautiously tested on humans.

Flower power

Clinical interest is such that it is easy to forget that RNAi is a natural process that operates in mammals as well as in lower organisms and plants. The mechanism probably evolved as a way to fight off pathogenic viruses. Many viruses have their genetic material made from RNA. So when they infect a cell, the RNAi pathway strikes back, shutting off key viral genes and aborting the infection.

The first clues of gene silencing were spotted in petunias in 1990. Dutch researchers were trying to produce more vibrantly coloured purple flowers by inserting extra RNA into normal plants. Instead, they lost pigmentation and turned white. Scientists were intrigued, though exactly what triggered these effects was not clear at the time.

A few years later, scientists discovered that the silencing mechanism is triggered by double-stranded RNA molecules, just 20 to 30 base pairs long, known as small interfering RNAs or siRNAs. These short strands target matching pieces of messenger RNA (mRNA) that contain the information necessary to manufacture a particular protein. Adding a few of these siRNAs to a cell disrupts that message. With no message there is no protein and the target gene shuts down.

Scientists soon found that it is relatively easy to create a piece of artificial RNA to trip up the cell’s mRNA machinery and turn off gene expression. They began exploiting RNAi to discover the function of thousands of genes.

This tool became so useful that in 2006 the Nobel prize for physiology and medicine was shared by US scientists Andrew Fire of Stanford University and Craig Mello of the University of Massachusetts, barely eight years after their discovery of the phenomenon in worms.

Strike back

It soon became obvious to biomedical scientists that, at least in theory, short snippets of RNA could be used to treat every disease imaginable. From bird flu to permanent hair removal, there are companies racing to harness RNAi’s tremendous therapeutic potential.

Ahead of the game is Alnylam Pharmaceuticals, of Cambridge Massachusetts. The company is entering Phase II studies with a treatment for respiratory syncytial virus, a childhood lung infection for which there is no treatment. If the strategy works, it could lead to a slew of anti-viral therapies.

RNAi could also be the ideal solution for the notoriously unpredictable HIV. Scientists already know that in the lab, a gene silencing mechanism prevents the virus’s replication in T cells, by cleaving its RNA and turning off its main proteins. But the virus mutates and evades resistance so rapidly that fighting it will take more than a single RNA target. ‘The virus always manages to escape from a single RNAi molecule, admits Karin von Eije from University of Amsterdam, the Netherlands. The team has been testing different short hairpin (sh)RNAs and their ability to stop viral replication in human T cells in culture. ‘It works well at first but eventually the HIV virus sneaks out. It mutates to become RNAi resistant,’ she says.

It takes four different types of RNA molecule, each designed to interfere with a different aspect of the virus, to stop HIV-1 escaping from RNAi. To turn this finding into a therapeutic, the researchers from the Berkhout lab in Amsterdam envisage taking progenitor cells from patient’s bone marrow and genetically modifying them with a lentivirus vector carrying the four therapeutic RNAs. The cells are then returned to the patient, who develops a healthy immune system protected against HIV.

Trials for HIV/AIDS using a similar strategy are taking place at the City

Nobel winners Andrew Fire (left) and Craig Mello (right) (seen here with a statue of Paul Ehrlich) discovered RNAi in worms

In short

- RNAi uses short interfering strands of RNA to silence specific genes in the cell
- The technology could be used to target a wide variety of diseases caused by aberrant genes including many types of cancer, diabetes and incurable neurodegenerative disorders
- It has shown huge promise in animal models but delivering RNAi as a therapy into target cells is the next hurdle for researchers

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of Hope biomedical research centre in California, US, directed by John Rossi, and in collaboration with Australian biotechnology company, Benitec.

Simone Hess, a molecular biologist at the Max Plank Institute for Infection Biology in Berlin, Germany, is bullish about this approach. ‘If the virus mutates too much, you can easily switch RNA sequences. It’s much faster than developing another small molecule,’ she says. Finding good drug candidates is costly and time consuming. If, at the end of that effort, the molecule fails, it’s back to the drawing board. By contrast RNAi relies on tweaking a few bits of RNA. ‘It is several orders of magnitude faster and more specific than conventional drugs,’ says Hess.

Topical RNAi applied to the genital tract could be especially effective. Judy Lieberman, senior investigator at Harvard Medical School, US has found that vaginal applications of siRNAs protect mice from a sexually transmitted infection – lethal herpes simplex virus type 2. An siRNA used as a microbicide could protect against viral infections with a similar infection route.

While siRNAs work well if delivered into tissues that are relatively easy to access, systemic delivery remains a vexing problem. The challenge is in getting them to cross the cell membrane. They need to be bound to liposomes, cholesterol, aptamers (small DNA molecules), chitosan or nanoparticles to get across and maintain high levels in the body (see p29). Flooding the system with RNA molecules does not work either – most particles remain in the liver.

By contrast, a guided missile strategy using antibody fusion works beautifully, says Lieberman. ‘You can deliver your drug specifically to the cells you want to target, you use less drug and it is less toxic.’ The Harvard researcher has fused an antibody fragment (for instance an antibody to LFA1, a protein manufactured by active T cells) to a fragment of protamine. While the protamine binds to siRNAs, the antibody discriminates between disease-activated T cells and resting T cells.

‘It’s an advantage when treating autoimmune disease or in transplant rejection, where you may not want to globally reduce the immune system, only to stop those cells causing the problem,’ she notes.

**Collagen carrier**

The idea of a simple switch-off is especially appealing in cancer, where tumours generally arise from aberrant genes. But unless the RNA therapeutic reaches every tumour cell and wipes it out, the cancer will return. Takahiro Ochiya, from the National Cancer Centre Research Institute in Japan, is pinning his involvement in human testicular germ cell tumours. Work by David Bartel at MIT suggests approximately 1000 human microRNAs regulate around one third of our genes.

MicroRNAs turn human genes on and off, not just individually, but as whole networks. So antagonising microRNAs could correct an entire disease pathway in a way that is impossible by today’s medicines. How microRNAs operate, however, remains largely mysterious. ‘Scientists are intrigued and challenged by microRNAs,’ says Urschel.

The field is exploding, but some results suggest that microRNAs could be constitutively expressed in a number of tissues, which could put a dampener on therapeutic efforts, as inhibiting microRNAs could lead to toxicity. ‘Everyone was so excited when it happened, but there are microRNAs everywhere, and people have realised it is very complex,’ says Susan Magdaleno, a scientist with RNAi company Ambion, in Austin, Texas.

While most agree that microRNA therapeutics set a new paradigm for treating disease, it may be some time before they can be fully exploited as treatments. ‘That’s the next big thing; elucidating exactly how microRNAs do it,’ says Urschel.
hopes on one formulation: the atelo-collagen molecule. This pepsin-treated collagen has all the attributes of an excellent drug carrier. ‘It’s charged, stable and well-accepted by the body. It resists digestion,’ he says.

In prostate cancer, atelo-collagen RNAi has yielded encouraging results. Bone metastasis is the main problem in the human disease, and Ochiya has created a luciferase (bioluminescent enzyme) mouse model to track progress. After a systemic injection, atelo-collagen-based RNA therapy rapidly disappears from all tissues, but it persists in the tumour for up to a week, probably due to poor lymphatic drainage. The Japanese researchers designed siRNAs for two prostate cancer genes to attach to the carrier. ‘It was a hit. Both those genes were good targets and there was no recurrence for two months.’

Ochiya has also silenced the breast cancer ‘slug’ gene with atelo-collagen RNAi. Although tumour growth was unaffected, the lymph nodes were clear – the cancer had not spread. ‘Slug could be a novel target to inhibit metastasis,’ he says.

**Forever young**

RNAs therapies could shut down troublesome genes that kick in with ageing. As baby boomers grow older, the toll of Alzheimer’s disease, for instance, is expected to rocket. The ideal therapy would stop beta-amyloid protein – associated with the formation of Alzheimer’s disease plaques – from accumulating in the brain. Xavier de Mollerat du Jeu, at Invitrogen in San Diego, US, has shown that, in a mouse model of the disease, injecting siRNAs (StealthTMRNAi) against the human amyloid precursor protein directly into the brain ameliorates the neurodegeneration. ‘The neurons took it up – we were very excited.’ But, the Invitrogen scientist stresses, ‘I don’t see grandma with a pump on her head. We are looking at some delivery reagents which will allow us to do a single injection rather than using a pump.’

RXi Pharmaceuticals is tackling ALS (Amyotrophic Lateral Sclerosis), a neurodegenerative disorder also known as Lou Gehrig’s disease. ‘ALS is rare but it’s gruesome, and there is nothing to help these patients,’ RXi’s Samarsky explains. A mutation in a gene called SOD1 triggers this devastating, progressive motor-neuron degeneration, leading to paralysis and death. So far, a pump delivering RNAi into the spinal column of a mouse ALS model shuts down up to 50 per cent of the harmful gene and extends lifespan by 20–35 per cent. It is a promising start. ‘When it works, it will be a great proof of principle for other neurodegenerative diseases – Alzheimer’s and Huntington’s, even pain. That’s our vision.’

At RXi, obesity and type 2 diabetes are also in the therapeutic firing line. Because the RIP140 gene is the body’s overall metabolic controller, it makes an excellent target. Mice genetically engineered to turn off RIP140 production are lean and resistant to diabetes even on a high fat diet. It’s a dream scenario for many consumers – to avoid a health risk commonly associated with eating a fatty diet, and even resist gaining weight. Traditional small molecule approaches have failed to tackle this key protein, but it may be treatable with RNAi. ‘It’s a dream scenario – to eat as much as you want without gaining weight’

**RNAi is effective in mouse models of chronic conditions including obesity and diabetes**

So how long will it be before the delivery issue is resolved? ‘Having it in every cell is probably not going to happen. But maybe you don’t want that,’ says Samarsky. He suggests that investigators should see whether any of the 110 different administration routes approved so far by the US Food and Drug Administration is good for their particular application.

Despite the hurdles, the RNAi field is set to grow aggressively. ‘It’s a completely different story’. RXi Pharmaceuticals could be in the clinic by 2009, and their arrival will shake up healthcare.

Set against the backdrop of the traditional, small molecule approach, Samarsky says RNAi is ‘a completely different story’.

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