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Editorial

RNA interference: a new therapy for neuropathic pain?

In 2006, Andrew Fire and Craig Mello were awarded the Nobel Prize for medicine for their pioneering work in developing techniques that allow a better understanding of gene function. Using segments of double-stranded RNA (dsRNA) injected into cells, they were able to show that the messenger RNA (mRNA) of a specific target gene could be effectively blocked or switched off. Using the tiny nematode *Caenorhabditis elegans*, they found that they could block the expression of specific genes whose nucleotide sequence was complementary to the sequence of the injected RNA [1]. This revolutionary method of gene silencing is now referred to as RNA interference (RNAi) [2].

The injected dsRNA molecule is broken down by the elegantly named enzyme, Dicer ribonuclease into a number of shorter sequences, 21–23 nucleotides in length. Each oligonucleotide, now called a 'short interfering RNA' (siRNA), is taken up into a protein complex where it is unwound and cleaved by means of an RNA helicase enzyme. The single-stranded siRNA now base pairs or hybridizes to the target mRNA, which it inactivates. Longer sections of dsRNA, although able to mediate gene silencing, can also trigger in mammalian cells an unwanted interferon response, which results in the widespread shut-down of protein synthesis. By designing short sequences of dsRNA against specific genes, researchers can exploit this novel concept and use siRNA as a genetic tool for inhibiting gene expression on a gene-by-gene basis without triggering unpredictable, off-target side-effects [3]. Using siRNA to disrupt genes that are specifically involved in disease represents a promising new method both to learn more about the role of the gene in the disease process and to quickly screen potential drug targets – a costly and time consuming aspect of drug development [4]. Furthermore, using siRNA therapeutically has become a distinct possibility and may help in the treatment of some intractable conditions where conventional therapy is lacking or remains ineffective [5]. An important example of such a condition is neuropathic pain.

Neuropathic pain is a debilitating, complex, chronic painful state that is usually accompanied by tissue injury and in particular, injury to the nervous system itself. The aetiology includes diverse conditions such as post-herpetic neuralgia, trigeminal neuralgia, diabetic neuropathy, spinal cord injury, cancer, stroke and degenerative neurological diseases. The pathophysiology of neuropathic pain is complex involving several pathways and receptors. Of pivotal importance, however, is the role of the *N*-methyl-D-aspartate (NMDA) receptor and its activation in the posterior horn of the spinal cord [6]. Prolonged or repeated activation of this receptor results in a cascade of neurochemical events, which manifest themselves as hyperalgaesia, allodynia and an increase in transmission for a given afferent input, which is referred to as 'wind-up'. In addition, and perhaps more significantly, there are thought to be changes in the genetic machinery leading to alteration in synaptic morphology. Recent work has suggested that there are electrophysiological changes in the NMDA receptor in peripheral nerve-injured neuropathic mice together with changes in the NMDA receptor subunits, which may underlie the hyperalgaesia [7].

Currently, the outcome of treatment of neuropathic pain is disappointing. Although there are a number of pharmacotherapeutic options, which include tricyclic antidepressants, antiepileptics and weak NMDA-receptor antagonists such as dextromethorphan and ketamine, the overall results from therapy provide patients with poor relief from symptoms, which may be both unrelenting and debilitating. In an attempt to evaluate more precisely the role of the NMDA receptor, researchers examined *in vivo* the effect of experimentally blocking the receptor using specific NMDA-receptor antagonists. They found that such a strategy was highly effective in increasing the efficacy of opiates in the experimental animal [8]. Furthermore, in animals there is clear evidence that when the NMDA gene was blocked by silencing the NR1

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subunit, there was significantly less pain compared with controls [9] and that there was markedly reduced associated neuropathic features such as hyperalgaesia and morphine tolerance [10].

The introduction of novel NMDA antagonists has unfortunately been limited until now by their unacceptable side-effect profile [11]. However, one new and exciting therapeutic option that will shortly be available is the targeting of the NMDA receptor using siRNA. Recently Tan and colleagues [12] targeted the gene, which encrypts the NR2 subunit of the NMDA receptor with specific siRNA injected intrathecally in mice. The result was potent, long-lasting silencing of the gene with abolition of formalin-induced pain behaviour. Similar results were obtained by Garaway [13] who demonstrated, also in mice, that injection of siRNA into the spinal cord dorsal horn, aimed at the NR1 subunit of the NMDA receptor, effectively achieved 75% knockdown of the receptor, which lasted for up to 6 months. This was accompanied by alleviation of symptoms of experimentally induced allodynia [13].

At present, the focus of attention has mainly been on the NMDA receptor as a conduit for the transmission of neuropathic pain. One other family of proteins currently under investigation are the transient receptor potential (TRP) group, which are expressed in the nervous system and which is involved in sensory physiology. One member of this family is the vanilloid type 1 receptor, which is encrypted by the TRPV1 gene. Using similar strategies of injecting siRNA intrathecally, Christoph and colleagues [14] were similarly able to demonstrate significant reductions in the levels of allodynia in a neuropathic rat model. Viscerally induced pain levels were also attenuated [14].

Only a few years after their discovery, RNAi has become an invaluable tool for researchers working in the field of functional genomics and target validation and has become the gold standard for studying loss of function phenotype, i.e. gene knockout models. This exciting new technology will shortly be within the reach of clinicians and already the number of possible applications, apart from the treatment of chronic pain syndromes, is rapidly growing. The potential therapeutic applications include the treatment of a variety of virus-induced diseases such as HIV, hepatitis, polio and influenza [15,16]. siRNA can also specifically inhibit oncogenes associated with leukaemia and has been found to have other potential roles in the treatment of neurodegenerative disease such as amyotrophic lateral sclerosis [17]. At present considerable effort is being made to develop appropriate vehicles to optimize the delivery and stability for siRNA. Both lipid-based delivery systems as well as vectors such as adenoviruses, retroviruses and plasmids have been examined. One promising avenue is the complexing of siRNA with polyethyleneimine (PEI), which both stabilizes the siRNA and releases it intact when injected systemically [18].

The discovery of RNAi has opened a window on the control mechanisms of intracellular protein production. Already pharmaceutical companies have begun to formulate strategies for harnessing this valuable therapeutic tool; indeed the first siRNA therapeutic substance Bevasiranib Sodium (Acuity Pharmaceuticals Inc., Philadelphia, PA, USA), intended for use in a common form of macular degeneration, has already successfully passed phase 2 trials. Despite the recent introduction of new classes of pain killers such as the N-type calcium channel blockers pregabalin and ziconotide, clinicians continue to be frustrated by neuropathic pain in their attempts to provide effective and reliable therapy. It is hoped that for the many patients whose lives are blighted by this unfortunate debilitating condition, siRNA will provide the much longed-for panacea.

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