Interfering with disease: opportunities and roadblocks to harnessing RNA interference

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RNA interference (RNAi) is an evolutionarily conserved mechanism for silencing gene expression by targeted degradation of mRNA. Short double-stranded RNAs, known as small interfering RNAs (siRNA), are incorporated into an RNA-induced silencing complex that directs degradation of RNA containing a homologous sequence. RNAi has been shown to work in mammalian cells, and can inhibit viral infection and control tumor cell growth *in vitro*. Recently, it has been shown that intravenous injection of siRNA or of plasmids expressing sequences processed to siRNA can protect mice from autoimmune and viral hepatitis. RNAi could provide an exciting new therapeutic modality for treating infection, cancer, neurodegenerative disease and other illnesses.

RNA interference (RNAi) is an ancient defense strategy that protects plants and lower invertebrates from viral infection and genomic damage by insertable genetic elements [1–5]. RNAi silences gene expression in a sequence-specific manner by cleaving mRNAs containing short sequences – 19 or more nucleotides in length – that are closely homologous to a target sequence. Two years ago, Sayda Elbashir and colleagues sparked a revolution in research when they demonstrated that RNAi operates in mammalian cells [6]. Researchers then moved rapidly to apply this new technique to understand mammalian gene function and to block gene expression *in vitro* [7]. A few studies have recently provided the proof of principle that RNAi can be used to protect mice from autoimmune and viral hepatitis. In this review, we will discuss the promise and hurdles in harnessing RNAi for human therapy and some of the clinical situations in which this novel therapy might be first applied.

RNAi was initially described in plants and in *Caenorhabditis elegans*, when it was discovered that double-stranded RNA (dsRNA) was much more potent at silencing gene expression than either sense or antisense single-stranded RNA (ssRNA) [1–4]. Feeding worms with short dsRNA homologous to a sequence in an endogenous gene caused a dramatic and specific reduction of gene expression [8]. In parallel, the phenomena of co-suppression of endogenous gene expression and the antiviral response in plants were understood to be due to post-transcriptional gene silencing of homologous sequences [9–11]. In the past few years, it has become clear that similar RNAi mechanisms exist in plants, fungi and throughout the animal kingdom, from worms and flies to mammals. RNAi works through the targeted degradation of mRNAs containing homologous (and in most cases identical) sequences to introduced short oligonucleotides (Fig. 1). In some organisms other than mammals, silencing can be amplified and spreads to target neighboring sequences via a process that involves an RNA-dependent RNA polymerase [12,13]. RNAi can also occur

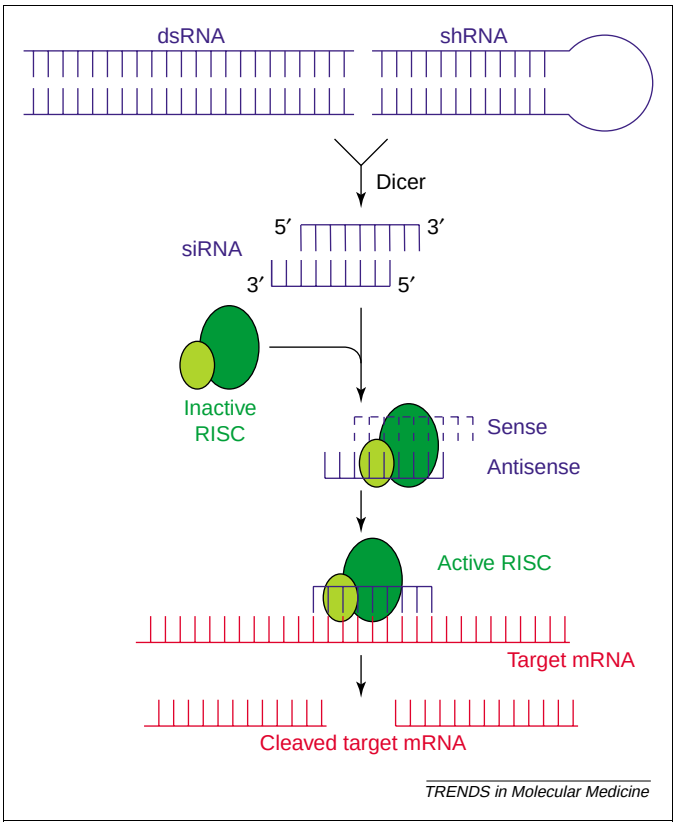

Fig. 1. Mechanism of RNA interference. Double-stranded (ds) RNA are processed by Dicer, in an ATP-dependent process, to produce small interfering RNAs (siRNA) of ~21–23 nucleotides in length with two-nucleotide overhangs at each end. Short hairpin (sh) RNAs, either produced endogenously or expressed from viral vectors, are also processed by Dicer into siRNA. An ATP-dependent helicase is required to unwind the dsRNA, allowing one strand to bind to the RNA-induced silencing complex (RISC). Binding of the antisense RNA strand activates the RISC to cleave mRNAs containing a homologous sequence.

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through additional mechanisms, which are just beginning to be described in mammalian cells [14]. These include some that work through changes in DNA methylation and chromatin structure induced by homologous dsRNAs, and others that work not through mRNA degradation but via translational arrest of mRNAs encoding closely homologous sequences [15–19]. These latter mechanisms use endogenous microRNAs (miRNA), which might be particularly important in regulating gene expression during development. miRNAs are often dsRNAs with imperfect pairing.

Intracellular generation of small interfering RNAs

In plants, small dsRNAs, complementary to both strands of the silenced gene, are generated from a longer dsRNA precursor [20]. RNAs of 21–22 nucleotides in length mediate RNAi, whereas longer sequences – 24 nucleotides in length – have been implicated in chromosomal silencing [21–23]. Processing of dsRNA to small interfering RNAs (siRNA) also occurs in *Drosophila* embryo and somatic cell extracts [24–27]. These endogenously generated siRNA have a unique dsRNA structure; each strand has a free 5′-phosphate end followed by 19 base pairs (bp) of paired RNA with a two-nucleotide unpaired overhang at the 3′ end (Fig. 1) [24,28]. The search for the enzyme that processes longer dsRNA into siRNA focused on the RNase III family of ribonucleases, whose substrates are duplex RNAs and whose products contain such 5′ ends. A class of evolutionarily conserved RNase-III-like enzymes, containing RNA helicase activity and sharing a common PAZ (piwi–argonaute–zwille) domain, has now been identified in plants and diverse animal species, and has been shown to process dsRNA into siRNA [29–31]. The siRNA-generating enzyme is called Dicer and is believed to act as a dimer. The RNAi machinery might also have other essential cellular functions; for example, deletion of RNAi genes in fission yeast interferes with proper segregation of chromosomes during cell division and maintenance of transcriptionally silenced heterochromatin [32–34]. Participation in important cellular functions could explain why there are no known examples of naturally occurring animal cells incapable of RNAi.

Mechanism of post-transcriptional gene silencing

The mechanism of gene silencing induced by siRNA, elucidated in plants, worms and flies, has only just begun to be described in mammalian cells. Initially in worms, RNAi was shown to act after transcription [35]. Using *Drosophila* embryo lysates, Phillip Sharp and colleagues then showed that silencing occurred via the destabilization of mRNA and suggested that dsRNA might trigger the assembly of a nuclease complex to target homologous RNAs for degradation [36]. The current model suggests that duplex siRNA are first unwound using an ATP-dependent helicase and then one strand of the siRNA is incorporated into a large precursor RNA-induced silencing complex (RISC) [27,37]. The activated RISC contains a single strand of RNA, a protein homologous to the product of the *Argonaute* gene family in plants (*rde-1* in *C. elegans*, *AGO2* in *Drosophila*, *GERp95* in human cells), and other uncharacterized factors, possibly including the unknown endonuclease responsible for cleaving the target RNA [37–40]. When the RNA in the RISC complex is complementary to a sequence in a target RNA, the RISC complex guides RNA cleavage in the middle of the homologous sequence.

In mammalian cells, the introduction of dsRNA >30 nucleotides in length into the cytosol activates a programme of global gene suppression by translational arrest, by activating the antiviral interferon (IFN) response [41–45]. Long dsRNA, produced in the life cycle of most viruses, activates the dsRNA-dependent protein kinase PKR, which phosphorylates the α-chain of the translation elongation αinitiation factor EIF2α, blocking all protein synthesis. As part of the antiviral response, activated PKR also induces IFN production and activates RNaseL to degrade RNA. However, because siRNA are only 21–25 nucleotides in length, they do not efficiently activate PKR or IFN production, and hence their silencing effects are exquisitely sequence-specific [28,46].

Gene silencing using RNAi in mammalian cells

Although RNAi is an important antiviral defense in plants, there is currently no evidence that the endogenous antiviral response in mammals uses RNAi. However, the description of RNAi in mammals is very recent. Viral inhibitors of RNAi have been described in a few plant and animal viruses [47], so it would not be surprising if some viruses that infect mammals and produce a dsRNA intermediate in the cytoplasm have devised self-protective strategies that involve inhibition of RNAi.

Delivery or expression of siRNA in mammalian cells has now been used to silence a wide range of genes in a variety of cell types [7]. These studies have included proof-of-concept studies to silence reporter genes (those encoding green fluorescent protein and luciferase) [6,48–56] and studies to knockdown gene expression as a rapid probe for gene function (e.g. those encoding tubulin, DNA methyltransferase, lamin A, cadherin, Cdc20 and Cdk2) [6,52,54,57]. An ever-increasing number of reports have probed potential therapeutic uses. For example, RNAi has been shown to work *in vitro* in a variety of conditions: (1) against cancer, by silencing *Bcr–Abl*, mutated *Ras*, human papillomavirus (HPV) E6 and E7, activating transcription factor 2, or overexpressed proto-oncogenes such as *Bcl-2* [58–62]; (2) against autoimmune diseases, by silencing Fas and caspase-8 [63,64]; against neurodegenerative diseases, by silencing polyglutamine sequences [65]; and (4) against viral infections with diverse replication strategies, including respiratory syncytial virus, poliovirus, hepatitis B (HBV), hepatitis C (HCV), HIV, HPV and influenza [66–77]. Even genes that are highly transcribed, such as those encoding lamin A and tubulin, have been effectively silenced. However, silencing is typically incomplete – a ‘knockdown’ rather than a ‘knockout’ – with residual gene expression usually at least a few per cent above baseline. Some residual gene expression might be due to untransfected cells in these studies. The studies on HIV successfully targeted a variety of viral genes (*gag*, *rev*, *tat*), as well as the viral long terminal repeat and host cellular receptors (CD4 and CCR5). In rapidly dividing transformed cells, silencing by transfected siRNA lasts for less

than a week, but transduced macrophages are effectively silenced for at least three weeks, at least for some targeted genes [78]. siRNA directed against HIV p24 block HIV production only if given within a week of infection, but provide sustained inhibition for at least three weeks in already infected cells.

Specificity of gene silencing

In the *Ras* study, it was possible to target mutated *Ras* without affecting unmutated *Ras*, demonstrating the exquisite specificity of RNAi as a therapeutic tool [58]. However, this specificity could pose problems for therapeutic uses in viral infection, where viral escape from RNAi selection in poliovirus has already been demonstrated [73], although the resistant virus was already present in the infecting inoculum in this study. One way around viral escape is to target more than one gene, either in the virus or by targeting both host and viral genes [68,78,79]. Moreover, the RNAi mechanism can tolerate some sequence mismatches, particularly away from the middle cleavage site [28]. However, this raises some questions about whether nonspecific silencing of partially homologous genes will present a problem for therapeutic use. In fact, a recent *in vitro* study showed that some genes with incomplete homology could be partially silenced, an effect that was more pronounced at higher concentrations of siRNA [80].

Vectored delivery of siRNA

Although these early studies were carried out by transfecting chemically synthesized siRNAs, more recently, dsRNAs have been expressed from stem–loop structures encoded by plasmids, retroviruses and lentiviruses [48,51–54,58,67,81–84]. These vectors provide a means for *in vivo* transduction of a variety of cells, including non-dividing cells, and open up a range of possible therapeutic uses for RNAi. The expression plasmids generally use a Pol III promoter and express sense and antisense strands separately or as a hairpin structure [48,51–54,58,67,81,82]. In cells, Dicer processes the short hairpin (sh) RNA [sense strand – short (~5–9 bp) loop – antisense strand; or alternatively, antisense strand – loop – sense strand) to an effective siRNA (Fig. 1). Sequences of siRNA expressed from retroviral and lentiviral vectors have been shown to silence expression effectively in a variety of primary cells [including embryonic stem (ES) cells, lymphocytes, macrophages and dendritic cells] [83,84]. Silencing can be controlled by engineering inducible expression of stem–loops [85]. Transfected or infected ES cells have already been used to produce transgenic mice with constitutive or inducible expression of siRNA [83]. However, infection with viral vectors could activate antiviral or inflammatory responses in some cells and interfere with the intended specificity. In particular, a recent study showed that shRNA vectors encoding siRNA, too short to induce an IFN response on their own, can nonetheless induce the expression of genes participating in the IFN response [80].

In vivo protection from hepatitis using RNAi

Although most of these studies have been performed *in vitro*, often with co-transfection protocols, which might not mimic natural conditions, a few recent studies have demonstrated *in vivo* silencing using RNAi. For example, rapid injection of duplex siRNA in a large volume (~1 ml) into the tail vein of mice effectively silenced co-injected reporter gene expression [55,56]. However, hydrodynamic injections are difficult to perform reproducibly in mice and require the rapid injection of 10–20% of the mouse blood volume. Therefore, it is unlikely that this method could be readily scaled up for clinical use. In the mouse experiments, the reporter gene was efficiently expressed only in the liver and a few other organs, and hence silencing was also demonstrated only in these organs. Hydrodynamically injected duplex siRNA directed against *Fas* were efficiently taken up by mouse hepatocytes (88%), and effectively silenced endogenous gene expression for ten days without diminution [63]. *In vivo* silencing in hepatocytes began to wane by 14 days and disappeared by 21 days. Mice with silenced *Fas* expression were protected from hepatic destruction and death in two models of autoimmune hepatitis (Fig. 2). The livers of *Fas*-siRNA-treated mice, but not of control mice, were dramatically

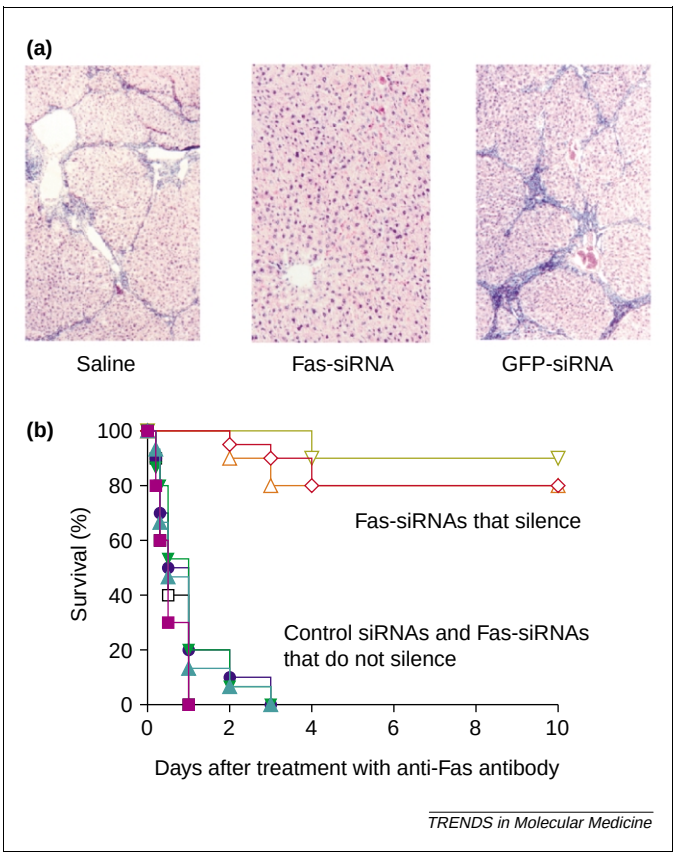

Fig. 2. *In vivo* protection from autoimmune hepatitis after high-pressure intravenous injection of small interfering RNAs (siRNA) targeting the apoptosis mediator, Fas. (a) Livers from mice given six weekly injections of concanavalin A were spared from fibrotic damage when Fas-siRNA was administered after the second and fourth injections (center panel). Injection of control saline solution (left panel) or of siRNA targeting the unrelated gene, green fluorescent protein (*GFP*) (right panel), did not prevent fibrotic damage. Original magnification × 100 for all panels. (b) In a more fulminant hepatitis model, induced by intraperitoneal injection of anti-Fas antibody, most mice pretreated with Fas-siRNA were protected from death, whereas all control mice died. Adapted from Ref. [63].

protected *in vivo* from treatment with concanavalin A (ConA) or anti-Fas antibody. In the former case, injections of siRNA could provide protection from hepatic fibrosis even when they were initiated after the second of six weekly injections of ConA. Anti-Fas siRNA protected mice from death induced by anti-Fas antibody; all control mice died within three days but 82% of *Fas*-siRNA-treated mice survived and had normal liver histology ten days later. Many forms of fulminant hepatitis, irrespective of etiology (viral, autoimmune or transplant rejection), are mediated by engaging the Fas receptor on hepatocytes, and hence silencing *Fas* expression or more distal mediators of apoptosis could be a useful strategy for treating hepatitis more generally. In fact, a recent study, silencing caspase-8 (activated downstream after Fas-receptor engagement), showed protection from both autoimmune and adenoviral hepatitis [64]. In addition, co-transfection by hydrodynamic injection in mice of an HBV plasmid [86] and a plasmid expressing shRNA targeting HBV sequences, processed to siRNA within cells, reduced HBV production and infection of liver cells [76]. Therefore, the potential of RNAi for treating hepatitis by targeting both apoptotic and viral genes has already been demonstrated *in vivo*.

Moving siRNA therapy from mice to people

Although hydrodynamic delivery of double-stranded siRNA can induce an effective silenced state in the livers of mice, it is unlikely that this strategy can be scaled up to humans because the introduction of duplex siRNA requires transiently generating high local intravascular pressure. In mice, siRNA are injected by bolus into the tail vein in a volume of ~10–20% of the mouse circulating blood volume. Although such a method could presumably be adapted to transduce selected tissues via regional catheterization [63,64] or to deliver siRNA pre-operatively into transplanted organs, it is unlikely to be possible to adapt this protocol safely for systemic human treatment.

Although dsRNA is resistant to most RNases, the intravenous half-life of unmodified duplex siRNA is very short, because the small molecules are degraded by endogenous nucleases and are quickly filtered through the kidneys. Therefore, duplex siRNA will need to be chemically modified for use as an injectable drug.

The usefulness of RNAi for therapy might be enhanced by targeted delivery to particular cells (such as virus-infected cells or tumors) or tissues, to prevent unwanted effects on normal host tissues. Targeted delivery can be achieved either by local administration (such as to the skin, mucosa or eye) or by injection via catheterization of the vascular supply to particular organs. In principle, chemically modified siRNA could also be covalently linked to ligands for cell-surface receptors, enabling delivery only to cells bearing these receptors. However, targeted delivery of duplex siRNA to induce a silenced state remains to be demonstrated. Similarly, viral vectors could theoretically be targeted by incorporating envelope genes for specific cell delivery.

Delivery strategies for therapy

Several strategies that have been developed to stabilize antisense oligonucleotides and ribozymes, including capping the 5′ ends, modifying the ribose sugars or substituting phosphorothioates, can stabilize siRNA without toxicity and might not jeopardize their ability to target mRNAs for degradation [87–89]. Moreover, some modifications of the sense strand, particularly at the 3′ end, are thought not to interfere with gene silencing [90]. Covalently coupling antibodies or ligands for cell-surface receptors to the 3′ end of the sense strand should provide a method for targeting siRNA specifically to cells or tissues of therapeutic interest. Covalent or even non-covalent linkage to basic peptides that deliver oligonucleotides into cells, or incorporation of siRNA into liposomes, could also provide a universal delivery mechanism [91,92]. Once inside the cell, duplex siRNA are expected to induce a silenced state that will last for 1–3 weeks (at least for cells that are not continuously undergoing cell division). This could be an ideal therapeutic window for many chronic diseases: long enough to require treatment only every few weeks or monthly, and short enough not to raise concerns about the long-term toxicity of permanently silencing a gene. However, introducing oligonucleotides into some non-dividing cells, such as lymphocytes or hematopoietic stem cells, is notoriously difficult and could require special strategies or delivery via viral vectors.

In addition to delivering siRNA as a drug, there is also considerable interest in delivering siRNA expressed from plasmids or viral vectors [48,51–54,58,67,76,79,81–84]. These approaches have all the benefits and drawbacks of gene therapy. The benefits are potential life-long expression of the siRNA, as a result of stable integration of the plasmid into genomic DNA, and the ability to target non-dividing cells such as stem cells, lymphocytes and neurons. The disadvantages are the low proportion of transduced cells and rapid loss of gene expression using current gene therapy methods, the danger of oncogenic transformation from insertional mutagenesis, and the danger of unanticipated toxicity from long-term silencing of human genes.

Therapeutic targets

Any gene is a potential target for silencing by RNAi, and hence the therapeutic possibilities are endless. This fact, coupled with the specificity and potency of silencing by siRNAs (~1000-fold more active on a molar basis than antisense oligonucleotides [93]), has caused some workers to tout siRNA as potentially the biggest revolution in therapeutics since the development of specific antibodies. Moreover, siRNA are likely to be much cheaper to manufacture and administer than antibody therapies. Therapeutic strategies for siRNA are based on silencing either ‘nonessential’ wild-type genes linked to disease or silencing mutated genes in which the mutation causes disease. Being able to deliver siRNA to specific cellular targets broadens the list of genes that can be silenced without inducing toxicity. Targeting mutated sequences is more costly and difficult to execute, because the therapy needs to be customized to the patient by determining the individual gene mutation and then synthesizing the homologous siRNA. The *in vitro* translational studies of RNAi carried out to date have focused mainly on viral infection and cancer, and these are likely to be the areas of early therapeutic efforts.

Viruses and other infectious agents

Although RNAi is an ancient antiviral defense in plants, it is unknown whether it plays any role in the natural defense of mammalian cells against viruses. Nonetheless, it seemed logical to try to harness this mechanism for the control of viral infection, particularly given that the silencing of viral genes is unlikely to have adverse consequences for the host. In searching for sequences to target, those that lack homology to human genes are used. RNAi could be used to treat acute viral infections, such as influenza and severe acute respiratory syndrome, which cause major morbidity and death, or to treat chronic infections that eventually progress. The chronic infections that cause the most morbidity throughout the world are HBV, hepatitis C (HCV) and HIV. Other intracellular pathogens, such as *Mycobacterium tuberculosis*, might also be amenable to RNAi. These are likely to be among the first therapeutic targets investigated.

Attractive viral targets are viral genes that are essential for replication, and sequences that are relatively conserved between viral strains, for which mutation would result in a less-robust infectious agent. Host genes that are required for viral entry or that play an essential role in the viral life cycle are also potentially good targets, providing that they are not required for survival of the cell. For RNA viruses, such as HIV or HCV, any region of the viral genome, including non-transcribed regions and regulatory genes, can also be targeted. It is possible that the RNAi machinery could be a limiting factor in mammalian cells, restricting the number of sequences that can be successfully targeted at the same time within a cell. Nonetheless, to minimize the risk of viral escape mutations undermining RNAi, the simultaneous targeting of several viral and/or host genes that are important at different stages in the viral life cycle might be a useful strategy. Moreover, synergistic enhancement of viral suppression has been demonstrated by targeting more than one gene [78].

Although bacteria are not amenable to silencing by siRNA, because they mainly replicate outside of cells and lack the RNAi machinery, it might be possible to reduce morbidity and mortality from life-threatening bacterial infections by silencing genes involved in those aspects of the stereotypical host immune response that lead to adverse consequences. For example, reducing the expression of pro-inflammatory cytokines, such as interleukin-1 (IL-1) or tumor necrosis factor α (TNF-α), might lessen the risk of septic shock, without jeopardizing the development of protective immunity. Such treatments would complement antibiotic therapies and could be instituted even before an infectious agent has been identified. In fact, in a recent study in mice, sepsis induced by treatment with lipopolysaccharide was blocked by injecting liposomes containing duplex siRNA targeting TNF-α [94].

Cancer

Several *in vitro* studies have already demonstrated the potential use of RNAi for treating cancers [58–61]. Gene targets that have been shown to slow tumor growth include the products of genes that are characteristically mutated generically or in specific cancers – either as translocations such as *Bcr–Abl* in chronic myeloid leukemia, or as point mutations such as *Ras* – to produce constitutively active oncogenes. The oncogenic potential of *Ras* mutation is atypical in that the changing of a single amino acid renders the molecule constitutively active and oncogenic, and hence a small number of different mutations are seen repeatedly in many cancers. As a result, targeting *Ras* would not need require a customized therapy for each tumor. However, although most of the other types of cancer mutations, such as translocations, occur in fairly well-defined locations, they are nonetheless too diverse to be targeted without sequencing the mutated gene for each tumor that needs to be treated. However, targeting the downstream sequence of a translocated gene, such as an *Abl* sequence in *Bcr–Abl*, might be an effective therapy, particularly if the tumor cell could be transfected selectively *in vivo*.

However, there are several genes that are not mutated in cancer, but are overexpressed to make cells resistant to native immunity, treatment or normal senescence. Many of these genes are overexpressed in a wide array of tumors and are attractive targets for silencing. These include the genes encoding: (1) the multidrug-resistance protein, MDR, which pumps chemotherapeutic drugs out of tumors; (2) telomerase, which overcomes the chromosomal shortening that occurs with each cell division; and (3) Bcl-2, which makes cell resistant to caspase-mediated apoptosis. Other potential targets for RNAi are growth factors or growth factor receptors that have an oncogenic role in specific tumors. Examples include IL-6 for multiple myeloma and Her-2 for breast cancer. Other possible targets are viral oncogenes, such as the HPV genes E6 and E7 for cervical cancer. Synthesis of angiogenic factors by vascular endothelia or tumors could also be silenced.

In fact, the list of possible cancer targets for RNAi includes all of the genes that have been recently targeted using conventional drugs, antibodies or antisense oligonucleotides. What makes RNAi an attractive alternative is the anticipated high degree of specificity of its effect, rarely seen with small-molecule inhibitors. The possibility of hitting multiple gene targets at the same time also greatly increases the chances of success. The success of RNAi therapy for cancer, as for other indications, will depend in large part on stable and, if possible, tumor-specific delivery. The delivery methods will need to be tailor-made for each type of cancer. For example, for cervical cancer, local instillation of stabilized duplex oligonucleotides could be all that is required. However, for disseminated or hematological tumors, targeting via cell-specific receptors might work better, particularly if the target gene(s) has an essential function(s) in non-transformed cells.

Neurodegenerative disease

Another class of diseases that have defied conventional approaches, but might be amenable to RNAi, are neurodegenerative diseases caused by the overexpression of mutated genes or of proteins containing long polyglutamine stretches [65]. Targeting the expression of the mutated allele or the polyglutamine sequence could slow or prevent the onset of neuronal cell death. Interfering with the expression of apoptotic genes selectively in the nervous system could have the same effect. It is not known

whether siRNA cross the blood–brain barrier. Again, the limiting factor is elucidating the best selective delivery strategy for targeting neurons. This might require local injection into the cerebrospinal fluid of modified siRNA or of retroviruses, or lentiviruses that efficiently infect nerve cells.

Other indications

The therapeutic possibilities are endless. Almost any disease in which expression or overexpression of a native or mutated gene causes disease is a potential target, providing that low expression (~10% of wild-type) will not be toxic. Initial clinical studies will inevitably be chosen to treat ailments that afflict a large number of people, but currently have no available treatment or a suboptimal or expensive treatment.

Potential sources of toxicity

SiRNA appear to have high specificity but, like any small molecule, both sequence-specific and -nonspecific impediments to therapeutic application could arise, once the delivery issues have been solved. Targeted delivery, if possible, will reduce the likelihood of toxicity. Most side effects are also expected to be dose-dependent and could be mitigated by administering less siRNA, adjusting its half-life by chemical modification, or by using a less-potent expression system for vectored delivery. Possible side effects include unintended silencing of partially homologous genes, either by mRNA degradation or suppressing translation, or induction of global gene suppression by activating the IFN response, especially when shRNAs are expressed from viral vectors. Another potential problem caused by the therapeutic introduction of siRNA could be inhibition of the function of endogenous miRNAs by competing for the RNAi machinery.

Conclusions

RNAi provides an exciting new therapeutic tool with a wide array of potential disease targets. The specificity and effectiveness of gene silencing with RNAi promises therapies with a high therapeutic index. Devising strategies to deliver siRNA into cells in a stable and cell-specific manner is the major impediment at present. Given the rapid pace in the field, with impressive demonstrations of efficacy *in vitro* and in mice, human pilot studies to provide the proof of principle for therapeutic use are likely to be undertaken within the next few years.

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