Ribonucleic Acid Interference: The Relevance of Molecular Targeting and Therapeutics to Neurosurgical Practice

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In October 2006, Andrew Z. Fire (Stanford University School of Medicine) and Craig C. Mello (University of Massachusetts Medical School) were awarded the Nobel Prize in Physiology or Medicine 2006 *for their discovery of RNA interference (RNAi)—gene silencing by double-stranded DNA* (http://nobelprize.org/).

We have compiled an overview of this new technology, addressing the basic concepts of ribonucleic acid (RNA) interference (RNAi), as well as several therapeutic applications for neurological disorders and its relevance for neurosurgeons. Encouraging results with RNAi-based therapies have been achieved in models of neurological disease, revealing the potential for translation to the treatment of neurodegenerative diseases, including movement disorders, brain tumors, and pain.[5,31] The clinical translation of this technology will have a direct impact into neurosurgery practice, requiring neurosurgical intervention for effective targeted delivery to the central nervous system (CNS).

Concepts: "The Central Dogma" and RNAi

Coincidentally, the Nobel Prize in Chemistry 2006, awarded to Roger D. Kornberg (Stanford University, CA) *for his studies of the molecular basis of eukaryotic transcription,* is closely related to the concept of RNAi. The theory of conversion of deoxyribonucleic acid (DNA) into protein, known as the "Central Dogma of Molecular Biology," includes the transcription (from DNA to messenger RNA [mRNA], that occurs in the nucleus-nucleolus) and the translation (protein synthesis using the genetic information from the mRNA, that takes place in the cytoplasm-ribosomes) pathways.

The RNAi gene-silencing process occurs at the post-transcriptional level (between the transcription and translation processes) through mRNA degradation, selectively blocking the expression of undesirable genes. Basically, a double-stranded RNA (dsRNA) that is homologous to a particular mRNA sequence enters the cell and an RNase-III enzyme called Dicer cleaves it into short double-stranded fragments of 21 to 23 nucleotides, containing 2-nucleotide 3′ overhangs. These small RNA sequences (small interfering RNA [siRNA]) are then incorporated into protein complexes called RNA-induced silencing complex (RISC). RISC uses the antisense strand of siRNAs as a guide to find and cleave target mRNAs. Finally, the cleaved mRNA is degraded, preventing translation and leading to gene silencing (*Fig. 19.1*).

The discovery of this mechanism was first published in 1998[9] in the nematode *Caenorhabditis elegans*. Thought to represent a conserved evolutionary mechanism against foreign RNA (plant viruses), RNAi evolved as an antiviral host defense mechanism. Using this natural process, the RNAi technology has been recently adopted as a means to study gene function and therapeutic silencing of gene expression.[27,40]

Types of siRNA

miRNAS

Micro RNAs (miRNAs) are inhibitory RNAs *naturally* expressed in cells during development and physiological processes. Asymmetric hairpin dsRNAs are cleaved by an enzyme called Drosha, producing precursor miRNA transcripts. Cleaved by Dicer, single-stranded mature transcripts are released and incorporated by RISC, which sequentially triggers the RNAi pathway.[15] miRNAs act not only at a posttranscriptional level, but also by inhibiting translation. Because of this natural defense feature, i.e., an endogenous mechanism to control gene expression that prevents rather than destroys target mRNA, miRNAs do not induce an immune response in mammalian cells.

siRNAs and shRNAs

siRNAs can be *artificially* constructed to knock-down gene expression. It has been demonstrated that synthetic siRNAs of 21 nucleotide RNA duplexes are enough to promote efficient and specific mRNA degradation in vitro.[7] Moreover, siRNAs shorter than 30 nucleotides do not trigger immune reactions in mammalian cells. Longer dsRNA molecules have been shown to cause an interferon response and activation of protein kinase R, with consequent nonspecific inhibition of transcription and translation.[23]

Because siRNA induces a transient effect, techniques have been developed to design synthetic siRNAs that are able

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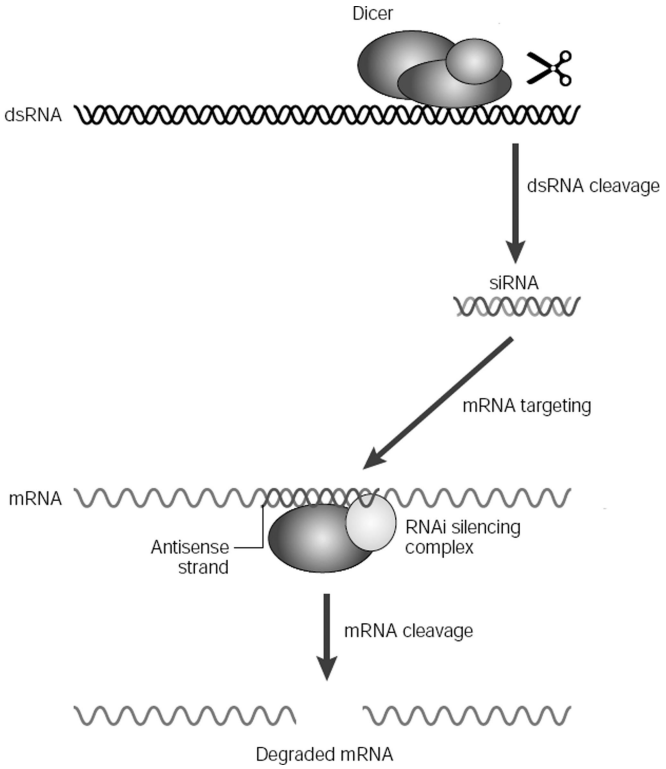

FIGURE 19.1. siRNAs. Long dsRNA is processed by Dicer to form siRNAs. The antisense strand of the siRNA is used by RISC to guide mRNA cleavage, promoting mRNA degradation. Adapted from McManus MT, Sharp PA: Gene silencing in mammals by small interfering RNAs. **Nat Rev Genet** 3:737–747, 2002.[24]

to promote long-term and stable gene silencing. shRNAs are synthetic forms of miRNAs. These single-stranded molecules assume a hairpin structure, which are recognized by Dicer and cleaved into functional siRNAs. They can be introduced into an expression vector system and processed to siRNAs in vivo. That is, the vector provides a means to deliver the gene encoding shRNA. Delivery of the gene results in sustained production of the shRNA in the targeted cell.

RNAi Expression Cassettes

Several methods can be used for inducing RNAi within mammalian cells, such as chemical synthesis and in vitro transcription, as well as cloning into plasmids and DNA vector systems for in vivo expression.

siRNA/shRNA expression cassettes, typically driven by pol III constitutive promoters (H1, U6, or 7SK), can be designed for inducing RNAi against any endogenous gene. Inducible regulation of RNAi, using systems such as tetracycline or ecdysone, however, may be more advantageous in cases in which the complete gene knock-down is unsuited or deleterious. Inducible systems have the ability to temporarily turn on or off gene expression, permitting a better control of the system.[1] More importantly, expression vectors can be designed to be allele specific, i.e., complementary to just one of the two copies of a gene inherited from the parents. RNAi libraries and database tools are available online to validate and optimize the design of target-specific expression cassettes and prevent nonspecific effects, i.e., the knock-down of normal (functional) alleles, instead of the desired selective silencing of pathogenic (mutant) alleles. Specificity of the RNAi therapy represents a major safety concern in the context of clinical translation.

DELIVERY

Nonviral Versus Viral Vectors

RNAi does not necessarily require a viral system for delivery. Several groups have demonstrated successful of RNAi using nonviral delivery systems, such as siRNA infusion and lipid-mediated approaches.[14,45] Nonviral delivery systems, however, have shown limitations in achieving lasting gene silencing, because of the transient effects of siRNA.[37]

Viral vectors, on the other hand, offer many advantages, particularly assisting the entry in the CNS. Moreover, they are able to confer specificity, and stable and controlled gene silencing. Lentiviral and adeno-associated viral (AAV) vectors have demonstrated the most efficient neuronal tropism and stable gene expression results in vivo.

Delivery to the CNS

Current methods of targeted drug delivery to the brain and the spinal cord include:

- *Intraparenchymal local* delivery through direct stereotaxic injection: this is a method of targeted delivery that circumvents the difficulty of transporting large molecules across the blood-brain barrier, but is invasive and, concerning intraspinal delivery, no validated strategies are currently available to safely achieve local delivery.
- *Intraventricular/intrathecal infusion* through the cerebrospinal fluid; *convection-enhanced delivery* via osmotic minipumps; *endovascular* (intravenous/intraarterial) injection by osmotic opening of the blood-brain barrier—although the three approaches permit the delivery of large volumes, targeted delivery is not guaranteed because of the widespread distribution of the drugs.
- *Remote injections:* a noninvasive alternative to conventional surgery, by taking advantage of the retrograde transport ability of neurotropic or engineered non-neurotropic viral vectors injected from peripheral sites (intramuscular and intraneural). Although successful in rodents, this approach has not been applied in neurosurgical trials and faces other challenges, such as the larger volume of vector needed to support the feasibility of this approach for human application.

Despite the therapeutic potential of siRNAs, adequate delivery remains a significant obstacle that still needs to be overcome. Combining neurosurgical drug delivery methods with improvements on expression of siRNA or shRNA will make the RNAi strategy more reliable for clinical translation.

CLINICAL APPLICATIONS

Diseases characterized by dominant mutation in a single allele of a gene are the best-suited candidates for RNAi therapy. However, despite progress, many neurological disorders still lack identification of genetic mutations. Gene silencing strategies can also target general pathways critical for disease progression, i.e., mutation-independent pathways. In this case, characterization of the physiological roles of involved genes is crucial to avoid the indiscriminate knock-down of ubiquitous or indispensable genes.

Herein, we will address neurological disorders that have either no therapies or only palliative options. For those, RNAi may represent a potential therapeutic option. Later, we will address a variety of diseases for which therapeutic options exist presently, but may benefit from RNAi-based strategies in the future.

Neurodegenerative Diseases

Alzheimer's Disease

Alzheimer's disease (AD), the major cause of dementia in the elderly, is characterized by a progressive accumulation of neurotoxic β-amyloid plaques in the brain. These plaques are composed of β-amyloid peptides, which are the metabolic product of the amyloid precursor protein (APP) after cleavage by β-secretase (also known as BACE1 [β-site amyloid precursor protein cleaving enzyme]).

Because BACE1 is considered to play a primary role in the pathogenesis of AD, strategies to silence BACE1 by RNAi have proven promising, using both nonviral and viral delivery systems.[18,28,36] Transfection of siRNAs targeted to BACE1 was able to successfully reduce the expression of APP and β-amyloid peptides in cultured cortical neurons from wild-type and APP transgenic mice.[18] In another study, Singer et al.[36] used intracerebral delivery of lentiviral vectors expressing siRNAs to specifically reduce BACE1 levels in the APP transgenic mouse model of AD. Moreover, they demonstrated amelioration in hippocampal neurodegenerative alterations and improvement in behavioral deficits.

siRNA-based strategies can also be applied to target other genes associated with the formation of amyloid plaques and intracellular neurofibrillary tangles in AD brains, such as *APP*, *neprilysin*, and *tau*.

Motor Neuron Diseases

Motor neuron diseases, including amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), constitute another group of progressive neurodegenerative diseases, characterized by upper and/or lower motor neuron degeneration, culminating in paralysis and death caused loss of airway control and respiration.

Familial ALS

Among the multiple etiologies proposed for ALS pathogenesis, involving motor neuron and glial cytotoxic mechanisms, 2% of ALS cases are associated with point mutations in the superoxide dismutase 1 (*SOD1*) gene, classified as familial ALS. Different groups have successfully achieved RNAi-based knock-down of the *SOD1* gene in animal models of ALS. Although these mutations affect only a small percentage of patients, selective silencing of the mutant *SOD1* copy may represent a promising therapy for familial ALS.

RNAi-mediated silencing of the *SOD1* gene was demonstrated in the SOD1 transgenic mouse model of ALS using lentiviral and AAV vector delivery systems. By intramuscular injection of a lentiviral vector based on the equine infectious anemia virus, Ralph et al.,[32] achieved success in silencing the *SOD1* gene in *SOD1*(G93A) transgenic mice, with consequent improvement in motor neuron survival (*Fig. 19.2*). At the same time, Raoul et al.,[33] using intraspinal vector delivery, also reported the ability of lentiviral vectors encoding SOD1 shRNA to reduce the expression of the *SOD1* gene. Both studies demonstrated substantial delay in the progression of the disease, as well as improvements in motor function and extended lifespan of SOD1 mice. Miller et al.[25] also showed the benefit of the siRNA therapy for ALS, by delivering siRNAs to spinal motor neurons through retrograde transport of AAV vectors injected into muscles.

More recently, Xia et al.[43] used a different approach to knock-down the *SOD1* gene. They generated transgenic mice

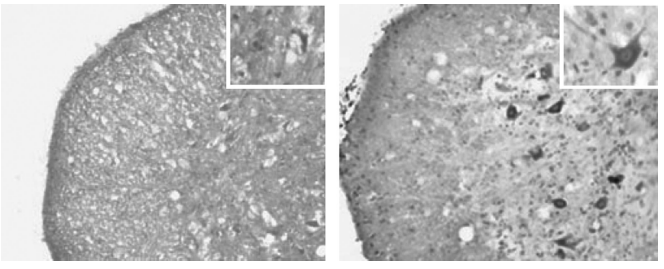

FIGURE 19.2. Motor neuron survival is improved by RNAi-mediated silencing of *SOD1*. Cresyl violet staining of lumbar spinal cord sections from lentiviral-injected mice at the end stage of disease (original low magnification, ×10; original high magnification, ×40). Survival of motor neurons was higher in lentiviral-SOD1-shRNA-injected mice (*A*) compared with control-injected mice (*B*). Reprinted from Ralph GS, Radcliffe PA, Day DM, Carthy JM, Leroux MA, Lee DC, Wong LF, Bilsland LG, Greensmith L, Kingsman SM, Mitrophanous KA, Mazarakis ND, Azzouz M: Silencing mutant SOD1 using RNAi protects against neurodegeneration and extends survival in an ALS model. **Nat Med** 11:429–433, 2005.[32]

that express an shRNA against the *SOD1*(G93A) mutation and crossed them with wild-type and SOD1 transgenic mice. The result was the specific silencing of the mutant *SOD1* allele but not the wild-type allele.

RNAi-mediated knock-down of genes involved in the apoptotic cascade are also being considered a potential alternative to avoid motor neuron degeneration in ALS. The prostate apoptosis response (Par)-4 protein is a mediator of neuronal degeneration associated with neurodegenerative diseases and highly expressed in the lumbar spinal cord of ALS patients. Xie et al.[44] demonstrated the specific silencing of prostate apoptosis response (Par)-4 by siRNAs, with consequent preservation of mitochondrial function and inhibition of caspase-3 activation in ventral spinal synaptosomes. They also confirmed the effectiveness of their strategy, showing protection from apoptosis of cultured spinal motor neurons from G93A transgenic ALS mice.

Spinal Muscular Atrophy

SMAs are autosomal recessive neuromuscular disorders characterized by loss of lower motor neurons, leading to muscle paralysis and atrophy. The disease is caused by homozygous deletion or mutations in one of two copies of the survival motor neuron (SMN) gene (*SMN1*) on chromosome 5q12, resulting in reduced production of the functional SMN protein. SMA is the most common genetic cause of infant death.

Although the SMN protein is ubiquitously expressed, it causes specific degeneration of motor neurons, representing a challenge to the understanding of the mechanisms of the disease. Mice have only one *SMN* gene, equivalent to the human *SMN1*, which is essential for embryonic development. Therefore, attempts to generate SMN knockout in mice have failed because of embryonic lethality.

Using the RNAi technology, Trülzsch et al.[39] have created an in vitro model to study *SMN* function and regulation. The authors used transient transfection of siRNAs to knock-down SMN expression in P19 cells, a cell line that differentiates into neuron-like cells. Surprisingly, siRNAs were able to transiently reduce both *SMN* RNA and protein levels, without affecting cell survival. This approach demonstrates the feasibility of the RNAi approach for further characterization of the *SMN* role in a neuronal context. By extension, this work demonstrates the application of vector-mediated RNAi to the creation of a variety of models for neurological diseases.

Polyglutamine Repeat Diseases

These autosomal dominant neurodegenerative diseases are characterized by a multiplication in CAG repeats (trinucleotide repeats) within the genes, leading to mutant expanded proteins (polyglutamine [polyQ] repeats).[8] Applying the concept of RNAi, specific inhibition of the mutant allele has provided a feasible approach to reduce these mutant proteins.[26] A variety of successful proof-of-principle data using transgenic mouse models for Huntington's disease (HD) and spinal cerebellar ataxia (SCA) have confirmed the therapeutic potential of the RNAi therapy, bringing optimism for its rapid future clinical translation.

Huntington's Disease

HD is an inherited neurodegenerative disease characterized by an abnormal polyQ repeat in exon 1 of the *huntingtin* (htt) gene on chromosome 4 that causes a toxic gain of function of the mutant expanded protein and consequent cortical and striatal degeneration.[8] Symptoms include cognitive dysfunction, involuntary movements (chorea), dementia, and psychiatric disturbance.

In vitro studies have provided confirmation of proof-of-principle for the ability of reducing the *htt* gene expression by RNAi. Silencing of htt in both nonneuronal and neuronal cell lines has been achieved by shRNA expression from both adenoviral nd lentiviral vectors.[12,17] Interestingly, adenovirus-mediated silencing of htt also reduced the formation of cellular aggregates.[17]

Reductions in htt mRNA and protein have been also shown using different transgenic mouse models of HD. Wang et al.[41] demonstrated the benefits of the RNAi strategy, silencing the *htt* gene expression in the brain of newborn R6/2 HD transgenic mice. By in vivo transfection, i.e., intraventricular injection of siRNAs, they inhibited transgenic htt expression in brain neurons and decreased the number of intranuclear aggregations in striatal neurons. In addition, the siRNA treatment delayed the onset of motor dysfunction and extended the longevity of R6/2 HD mice. In another study, recombinant AAV1 vectors expressing shRNAs were injected into the striatum of HD-N171–82Q mice, reducing human htt transgene levels by approximately 50% compared with control animals. Moreover, *htt* gene silencing led to significant benefits on motor and neuropathological abnormalities in these transgenic mice.[13] Similarly, Rodriguez-Lebron et al.[34] demonstrated an important reduction in the mRNA levels of mutant *htt* and protein, as well as reduction of neuronal intranuclear inclusions and behavioral improvements in the R6/1 HD transgenic mouse model. In this case, they injected rAAV5-shRNAs vectors into the striatum of R6/1 transgenic mice, achieving long-term in vivo expression of anti-mHtt shRNAs. More recently, Machida et al.[22] showed that AAV5-mediated intrastriatal delivery of RNAi improved HD pathological abnormalities in the HD190QG mouse model of HD, even after the onset of disease. Although other examples of RNAi approaches to HD exist, these applications are the most effective results achieved to date.

Spinal Cerebellar Ataxia

SCA Types 1 and 3 are dominant polyQ disorders that result in intranuclear aggregates, containing abnormal

ataxin-1 and -3. Progressive ataxia, cerebellar atrophy, and death of Purkinje cells are the common findings.

Using SCA1 transgenic mice, which have the specific expression of the human mutant allele ataxin-1 restricted to cerebellar Purkinje cells, Xia et al.[42] demonstrated the efficacy of AAV1.shSCA-1 vectors in silencing ataxin-1 (*Fig. 19.3*). Through middle cerebellar lobule vector injections, this approach was able to restore cerebellar morphology, reduce intranuclear inclusions, and improve behavioral in SCA1 mice.

Allele-specific silencing has been demonstrated in Machado-Joseph disease, also known as SCA3.[26] By targeting a single-nucleotide polymorphism in the *MJD1* gene, which codes ataxin-3, the authors designed siRNAs, containing G-to-C mismatches in different positions and analyzed in vitro specific inhibition of the mutant allele. They also constructed expression plasmids and recombinant adenoviral vectors expressing the most efficient allele-specific siRNA and, again, demonstrated independent wild-type or mutant ataxin-3 silencing in differentiated PC12 cells that inducibly expressed normal or mutant ataxin-3.

Movement Disorders

DYT1 Dystonia

DYT1 dystonia or early-onset primary dystonia is the most common hereditary form of generalized dystonia. DYT1 is a dominant inherited movement disorder characterized by a 3 basepair deletion in the *DYT1* gene, which leads to abnormal accumulation of mutant torsinA protein. Overexpression of torsinA is associated with abnormal nuclear envelope membranes, resulting in perinuclear inclusions. Symptoms of DYT1 include involuntary muscle contractions and abnormal posture.

In vitro studies have demonstrated the potential of viral-mediated RNAi for blocking mutant torsinA as a promising therapy for DYT1. Using lentiviral-mediated delivery of shRNAs targeted specifically against the mutant allele, two distinct groups achieved allele-specific knock-down of the mutant torsinA, restoring the normal distribution of the wild-type protein.[11,19] Interestingly, Gonzalez-Alegre et al.[11] achieved silencing of torsinA without immune response activation.

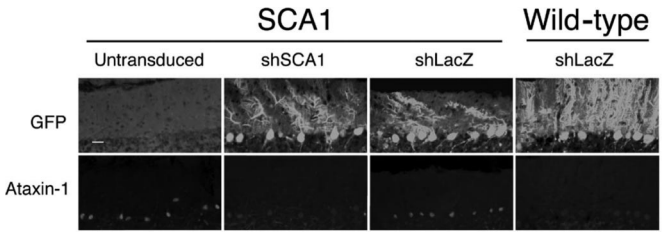

FIGURE 19.3. RNAi reduces intranuclear inclusions in transduced cells. Brains from SCA1 and wild-type mice were harvested 9 weeks after gene transfer (16 weeks of age) and processed to evaluate human recombinant green fluorescent protein (GFP) fluorescence and ataxin-1 immunofluorescence. Scale bar, 25 μm (representative of all images). Reprinted from Xia H, Mao Q, Eliason SL, Harper SQ, Martins IH, Orr HT, Paulson HL, Yang L, Kotin RM, Davidson BL: RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia. **Nat Med** 10:816–820, 2004.[42]

Parkinson's Disease

Parkinson's disease (PD) is a neurodegenerative disorder characterized by a progressive loss of dopaminergic nigrostriatal neurons and formation of α-synuclein inclusions, also known as Lewy bodies. Clinical symptoms include bradykinesia, rigidity, resting tremor, and postural instability. Although dopaminergic drugs provide relief for the majority of patients, cell and gene therapies are emerging as neurorestorative alternatives for those patients refractory to conventional treatments.

Although the pathogenesis of PD is not completely understood, there is strong evidence for the involvement of α-synuclein, because of its overexpression and accumulation in Lewy bodies.[21] Recent in vitro and in vivo studies achieved RNAi-mediated knock-down of α-synuclein. By using siRNAs targeted to endogenous α-synuclein, Fountaine and Wade-Martins[10] showed a reduction of 80% in the protein level in neuroblastoma cells. Moreover, they demonstrated that the suppression of α-synuclein was related to decrease in dopamine transport. Sapru et al.[35] used lentiviral vectors expressing shRNAs to target the human α-synuclein gene. Their results showed potent reduction (90%) of endogenous α-synuclein protein in human SH-SY5Y cells and in rat brain.

The RNAi technology, on the other hand, might help to elucidate the pathogenic mechanisms of PD. Hommel et al.[16] demonstrated specific knock-down of the *Th* gene, which encodes tyrosine hydroxylase, the enzyme responsible for dopamine production, within neurons of the substantia nigra pars compacta of adult mice by stereotactic injection of AAV-shRNA vectors. *Th* gene silencing resulted in behavioral deficits consistent with PD, which might help the development of future PD animal models.

Acquired Diseases

Brain Tumors

Because standard treatments, including surgery, radiation, or chemotherapy, have shown limited improvements in prolonging survival of patients, gene therapy and RNAi strategies might be promising as novel therapeutic options for malignant brain tumors. A series of in vitro and in vivo studies have confirmed the potential of both strategies. The main focus of ongoing RNAi-based strategies is the silencing of oncogenic genes to confine apoptosis within tumor cells. Inhibition of tumor angiogenesis and molecules that play a

role in tumor survival also represent promising molecular targets.

The human epidermal growth factor receptor (EGFR) plays an oncogenic role in solid tumors and is expressed and dysregulated in 90% of brain tumors. Different groups have tried to silence EGFR via the RNAi-based strategy. Using weekly intravenous administration of polyethyleneglycol (PEG) immunoliposomes (PILs) carrying shRNA plasmids against EGFR, Zhang et al.[46] reported a reduction of EGFR expression in tumors and increase lifespan of *scid* mice that had U87 glioma cells implanted. PIL is a lipid-mediated delivery system for plasmid-based siRNAs. Briefly, the surface of liposomes is conjugated with strands of PEG, forming pegylated liposomes. Then, the PEG strands are conjugated with receptor-specific monoclonal antibodies, forming the pegylated immunoliposome. The targeting monoclonal antibody enables the PIL to bind to specific endogenous cell surface receptors located in the brain vascular endothelium.

Intravenous RNAi therapy increased survival in 88% in mice with advanced intracranial brain tumors (*Fig. 19.4*).[46]

Human Immunodeficiency Virus-induced Encephalopathy

The RNAi technology can be used as a general antiviral tool against viral encephalopathies, such as the infection caused by the human immunodeficiency virus type (HIV)-1.[30] Either cellular co-receptors or viral proteins can be targeted for gene silencing. For instance, RNAi can be used to delete or reduce expression of the human *CCR5* gene, which encodes a co-receptor for HIV entry into cells.[2] siRNAs designed to recognize both murine and human CCR5 mRNA were able to effectively reduce CCR5 transcripts and protect cells in vitro. siRNA-mediated knock-down of sequences conserved in neurotropic strains of HIV-1 is also an effective approach to inhibit HIV-1 infection in the CNS that has been shown to be feasible in primary human macrophages.[4]

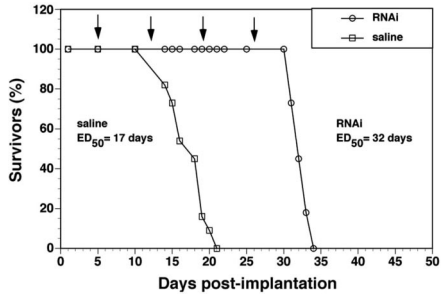

FIGURE 19.4. RNAi-mediated increase in survival. Intravenous RNAi gene therapy directed at the human EGFR is initiated at 5 days after implantation of 500,000 U87 human glioma cells in the caudate putamen nucleus of *scid* mice, and weekly intravenous gene therapy is repeated at Days 12, 19, and 26 (*arrows*). The control group was treated with saline on the same days. There are 11 mice in each of the two treatment groups. The time at which 50% of the mice were dead (ED_{50}) is 17 days and 32 days in the saline and RNAi groups, respectively. The RNAi gene therapy produces an 88% increase in survival time, which is significant at the $P < 0.005$ level (Fisher's exact test). Reprinted from Zhang Y, Zhang YF, Bryant J, Charles A, Boado RJ, Pardridge WM: Intravenous RNA interference gene therapy targeting the human epidermal growth factor receptor prolongs survival in intracranial brain cancer. **Clin Cancer Res** 10:3667–3677, 2004.[46]

Pain

RNAi is a potential tool to silence molecular targets involved in conditions such as chronic inflammatory and neuropathic pain, which are particularly resistant to conventional treatments. Pain receptors, including the P2X3 receptor, the vanilloid receptor VR1, and opioid receptors, are involved in pain perception and form potential targets for RNAi-based interventions to provide analgesia.

The P2X3 receptor localized in dorsal root ganglion neurons plays a role in sensory signaling, modulating neuropathic pain sensation. Intrathecal siRNA infusion can specifically knock-down the rat P2X3 receptor. Down-regulation of P2X3 in dorsal root ganglion and spinal cord consequently resulted in relief of chronic pain and behavioral reduction of allodynia and mechanical hyperalgesia in the partial sciatic ligation model of chronic neuropathic pain.[6]

RNAi-mediated knock-down of the EP4 prostanoid receptor, which is a subtype of prostaglandin E_2 receptors, has also been used for the study of its function in inflammatory pain. Intrathecal delivery of shRNA against the EP4 receptor was able to block the pain response and attenuate inflammatory pain hypersensitivity in a rat model for inflammatory pain induced through intradermal injection of complete Freund's adjuvant.[20]

Prion Diseases

Prion diseases, also known as transmissible spongiform encephalopathies (TSEs), are a group of human and animal fatal neurodegenerative diseases characterized by spongiosis, neuronal death, and astrogliosis. The pathogenesis of prion diseases is characterized by the accumulation of the abnormal isoform (PrPsc) of the host-encoded prion protein (PrPc) in the CNS. Creutzfeld-Jakob disease is the main human form of TSE, and bovine spongiform encephalopathy is the form found in cattle. Variant Creutzfeldt-Jakob disease is the human form resulted of exposure to the agent that causes bovine spongiform encephalopathy. Scrapie is the disease associated with the ovine prion protein.

Investigators have successfully tested the hypothesis of RNAi-based silencing of the *Prnp* gene that encodes PrPc as a therapeutic approach for treating TSEs.[3] Two different groups reported in vitro down-regulation of *Prnp*. Tilly et al.[38] achieved specific and efficient reduction in PrPc levels in RK13 transfected cells expressing the ovine *Prnp*. In another approach, the authors demonstrated *Prnp* gene silencing in

scrapie-infected neuroblastoma cells, corroborating the therapeutic potential of RNAi for prion diseases.[3]

Recently, in a very elegant study, lentiviral shRNAs were used to transduce embryonic stem cells and generate chimeric mice. Depending on the degree of chimerism, the mice carried a different level of the lentiviral shRNAs in brain cells and expressed reduced levels of PrPc. After scrapie infection, highly chimeric mice demonstrated extended survival compared with control animals.[29]

CONCLUSIONS

We have tried to present an overview of recent advances of the RNAi technology and its relevance to the neurosurgical practice. On the basis of the concept of this strategy, we first demonstrated how RNAi provides a means to target the activity of mutant genes. Challenges to the future success and clinical translation of this potential therapy, including allele specificity and delivery systems, were discussed. Finally, by illustrating its application in neurological disorders derived from the activity of specific mutations in genes, we highlighted the importance of RNAi-based therapies for neurosurgeons and their role in the clinical translation of this technology.

The approaches discussed herein achieved optimistic and encouraging results, which may offer an attractive option for the treatment of neurological disorders, particularly in the case of HD and malignant brain tumors. Efforts have been undertaken to improve siRNA specificity, delivery, and performance.

RNAi-mediated therapy might not be sufficient alone for the majority of diseases discussed in this review. However, the silencing of single molecular pathways opens new avenues for the understanding of disease processes and future therapeutic intervention.

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