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RNA therapy for polyglutamine neurodegenerative diseases

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Polyglutamine neurodegenerative diseases result from the expansion of a trinucleotide CAG repeat, encoding a polyglutamine tract in the disease-causing protein. The process by which each polyglutamine protein exerts its toxicity is complex, involving a variety of mechanisms including transcriptional dysregulation, proteasome impairment and mitochondrial dysfunction. Thus, the most effective and widely applicable therapies are likely to be those designed to eliminate production of the mutant protein upstream of these deleterious effects. RNA-based approaches represent promising therapeutic strategies for polyglutamine diseases, offering the potential to suppress gene expression in a sequence-specific manner at the transcriptional and post-transcriptional levels. In particular, gene silencing therapies capable of discrimination between mutant and wildtype alleles, based on disease-linked polymorphisms or CAG repeat length, might prove crucial in cases where a loss of wild type function is deleterious. Novel methods, such as gene knockdown and replacement, seek to eliminate the technical difficulties associated with allele-specific silencing by avoiding the need to target specific mutations. With a variety of RNA technologies currently being developed to target multiple facets of polyglutamine pathogenesis, the emergence of an effective therapy seems imminent. However, numerous technical obstacles associated with design, discrimination and delivery must be overcome before RNA therapy can be effectively applied in the clinical setting.

Neurodegenerative diseases represent a significant proportion of the noncommunicable disease burden in developed countries, for which few treatment options are available. A subset of these conditions, collectively known as polyglutamine (polyQ) diseases, have a well-defined genetic aetiology, making them attractive targets for gene-based therapies. There are currently nine known inherited polyQ diseases: Huntington's disease (HD),

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dentatorubral-pallidoluysian atrophy (DRPLA), spinal bulbar muscular atrophy (SBMA) and six spinocerebellar ataxias (SCA1, 2, 3, 6, 7 and 17). All these conditions arise from an expanded trinucleotide CAG repeat within the coding region of the disease-causing gene, which is translated into an abnormally long polyQ tract in the mutant protein (Ref. 1). The length of the CAG repeat is prone to expansion from one generation to the next (Ref. 2) and shows a strong correlation with both age of onset and disease severity (Refs 3, 4, 5, 6).

Symptoms, including chorea, dystonia and dementia (HD), ataxia and dysarthria (SCAs and DRPLA), retinal degeneration (SCA7), seizures (SCA17) and motor weakness (SBMA), result from the selective degeneration of well-defined subpopulations of neurons (Refs 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18). Although the precise mechanisms underlying this selectivity remain incompletely understood, the identification of common hallmarks of pathology suggests that many, if not all, of the polyQ diseases may be amenable to similar therapeutic approaches.

One such common feature is the formation of neuronal intranuclear inclusions (NIIs), which result from the increased aggregation propensity of expanded polyQ protein regions, and might involve the sequestration of additional components, such as transcription factors and heat shock proteins (Refs 5, 19, 20). The role of these NIIs in pathogenesis remains controversial however, with some studies suggesting a protective role for the sequestration of mutant polyQ protein in inclusion bodies (Refs 3, 21). Numerous additional mechanisms have therefore been suggested to explain polyQ-expansion-related pathogenesis (Fig. 1) (Refs 3, 22, 23, 24). These include altered transcriptional regulation by polyQ-expanded proteins (Refs 20, 25, 26, 27, 28), abnormal processing of the mutant protein (Refs 3, 29, 30, 31, 32), mitochondrial dysfunction (Refs 18, 33) and proteotoxic stress, caused by impairment of the ubiquitin–proteasome pathway (Refs 34, 35, 36).

All these mechanisms represent potential therapeutic targets; however, the most effective and widely applicable therapies are likely to be those designed to target and suppress production of the mutant protein upstream of these deleterious effects (Ref. 37). RNA-based approaches, such as RNA interference (RNAi) and antisense oligonucleotides (AONs), represent promising therapeutic strategies for polyQ diseases because they offer the potential to target genes in a sequence-specific manner, regardless of known protein function (Refs 38, 39). However, novel technologies such as transcriptional gene silencing (TGS) are gaining credibility and might offer alternative mechanisms to specifically and sustainably silence the mutant gene. This review focuses on the most recent applications of these technologies and examines the crucial considerations that must be taken into account when assessing the applicability and clinical utility of such therapies.

RNA-based therapies for polyQ diseases

Because polyQ diseases are caused by a toxic gain of function of the mutant protein, it follows that suppression of expression of the mutant gene containing the pathological CAG expansion should halt progression of the disease (Ref. 40). Indeed, several lines of evidence support the therapeutic benefit of silencing polyQ-encoding genes (Ref. 41). In particular, studies of inducible models of disease have demonstrated that neuropathological features, which develop over time in response to the presence of expanded polyQ protein, are rapidly improved once expression of the mutant gene is attenuated. Such findings provide proof of principle that inhibition of disease-causing genes may represent an effective means of therapy in patients already exhibiting a disease phenotype (Refs 42, 43). Thus, attention has turned in recent years to the development of effective transcriptional and post-transcriptional gene silencing therapies, mediated for the most part by small noncoding RNA molecules (Fig. 2).

The most straightforward method of RNA-based gene silencing therapy involves indiscriminate suppression of both the normal and pathological alleles of the gene, termed 'non-allele-specific silencing' (Fig. 2b). Although technically less challenging, this approach assumes that the wild-type function of the gene is either nonessential or functionally redundant, because silencing of genes essential for central nervous system (CNS) development and maintenance could have deleterious consequences.

In cases where wild-type protein function is indispensable, an allele-specific approach might be required. Allele-specific silencing relies on the

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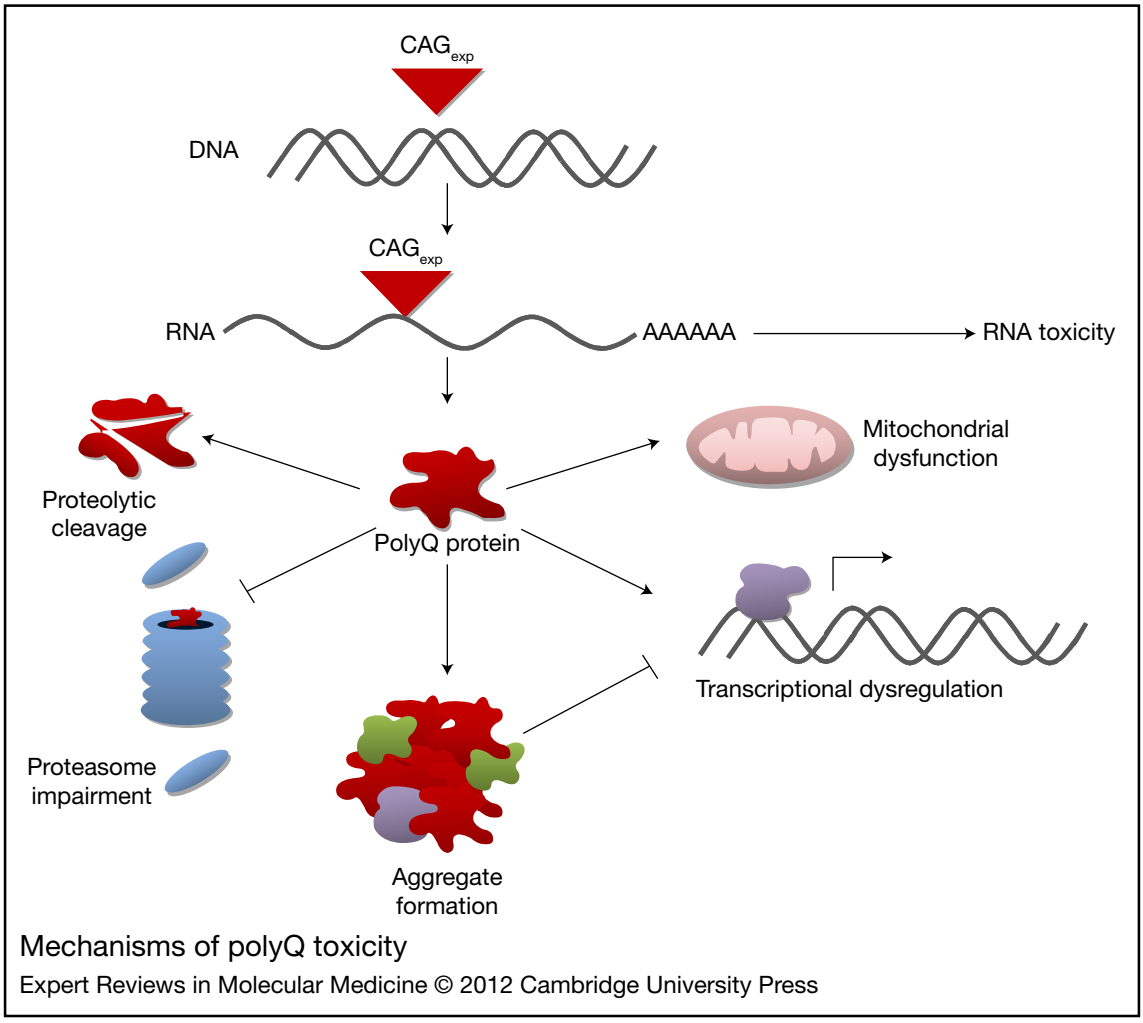
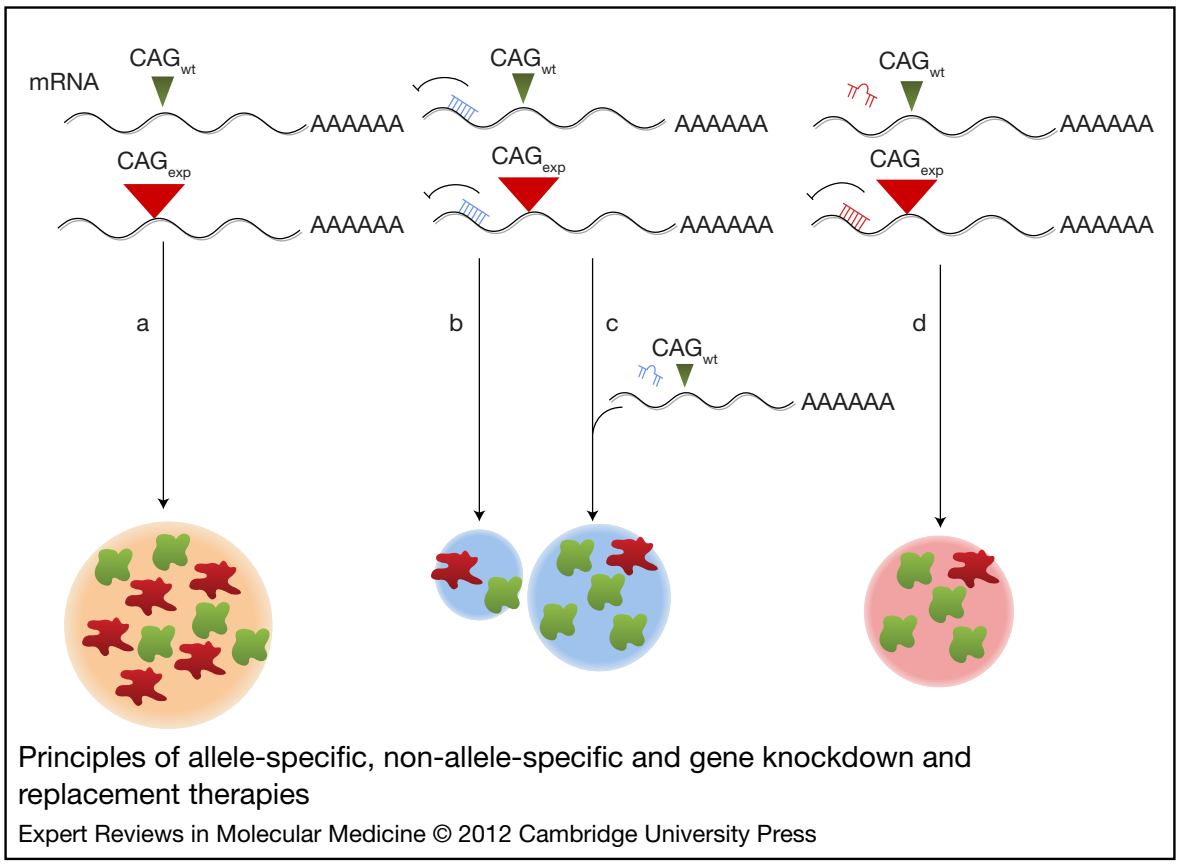


Figure 1. Mechanisms of polyQ toxicity. The expansion of CAG trinucleotide repeats within the coding region of several genes results in the expression of proteins containing pathologically expanded polyglutamine (polyQ) tracts. Pathogenesis has largely been attributed to the effects of the mutant protein, although a role for RNA toxicity in the development of disease has also been proposed. The mechanism by which polyQ proteins exert their toxic effects varies according to protein context, and may include proteolytic cleavage (leading to the production of toxic fragments), impairment of the ubiquitin–proteasome pathway, formation of aggregates of mutant protein (involving the sequestration of wild-type polyQ protein and other important cellular components such as transcription factors), dysregulation of transcription, either directly or as a result of aggregate formation, and mitochondrial dysfunction, all of which result in deleterious downstream consequences.

availability of targetable sequence differences that enable discrimination between the normal and pathologically expanded transcripts of a gene, such as single-nucleotide polymorphisms (SNPs) inherited in linkage disequilibrium with the expansion mutation, or differences in the CAG repeat length itself (Fig. 2d) (Ref. 44). In theory, an allele-specific strategy represents the preferred therapeutic mechanism for most polyQ diseases, because it allows selective silencing of the mutant copy of the gene while maintaining wild-type expression. However, in practice, it is not always feasible: polymorphisms linked to the disease allele in a small number of affected individuals may not justify the development of a therapy and may not be amenable to targeting because of their location within the gene (Ref. 45). In such cases,

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Principles of allele-specific, non-allele-specific and gene knockdown and replacement therapies

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Figure 2. Principles of allele-specific, non-allele-specific, and gene knockdown and replacement therapies. (a) Translation of wild-type and pathologically expanded alleles of a CAG repeat results in the production of both mutant (red) and wild-type (green) protein. (b) Non-allele-specific post-transcriptional gene silencing (TGS) therapy (shown in blue) prevents translation of both transcripts, leading to a reduction in wild-type and mutant polyglutamine protein levels. (c) In the case of gene knockdown and replacement, the introduction of an exogenous wild-type transcript, which is resistant to silencing as a result of modifications to the nucleotide sequence, results in restoration of wild-type protein expression despite the suppression of both endogenous transcripts in a non-allele-specific manner. (d) Allele-specific gene silencing (shown in red), based on discrimination between single-nucleotide polymorphism genotypes or differences in CAG repeat lengths, results in selective suppression of mutant protein production, while wild-type protein levels remain relatively unchanged. Effectors may be either RNA interference-based or antisense oligonucleotides.

a novel approach known as gene knockdown and replacement may be used. This method combines non-allele-specific silencing with gene replacement therapy, in which an RNAi or AON-resistant transgene is delivered to the targeted tissues together with the therapeutic effector (Fig. 2c) (Refs 46, 47).

The decision as to which silencing method to apply to a given polyQ disease depends on both the degree to which loss of wild-type protein expression is tolerated and the feasibility of allele-specific silencing. To date, answers to these questions have largely been based on the phenotypes observed in knockout and transgenic animal models of disease (Table 1). In the case of HD, for instance, *HTT* knockout results in embryonic lethality (Ref. 48), whereas conditional knockout mice display a neurodegenerative phenotype and a significantly shorter lifespan (Ref. 49). Ablation of wild-type function in transgenic HD mice results in an exacerbation of disease symptoms (Ref. 50). Because *HTT* is known to have a role in several key pathways in brain development,

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retention of wild-type expression appears necessary, although the absence of a phenotype in heterozygous conditional knockout mice and in an individual expressing *HTT* at only 50% of normal levels suggests that a single functional copy may be sufficient (Refs 51, 52) – a strong argument in favour of the allele-specific silencing approach.

Table 1. PolyQ gene function, phenotypic effect of loss of function, and implications for choice of gene silencing therapy

PolyQ disease	Gene affected	Function of wild-type protein	Knockout model phenotype	Preferred therapy
HD	*HTT*	Transcription, intracellular signalling, trafficking, endocytosis, metabolism (Refs 7, 153)	Embryonic lethality (Ref. 48), conditional knockout shows progressive neurodegeneration and sterility (Ref. 49)	Allele-specific, non-allele-specific shows promise
DRPLA	*ATN1*	Nuclear receptor corepressor (Ref. 154)	No phenotype (Ref. 60)	Non-allele-specific
SBMA	*AR*	Nuclear receptor, ligand-dependent transcription factor (Ref. 63)	Severe testicular feminisation and infertility (Ref. 62)	Gene knockdown and replacement
SCA1	*ATXN1*	Gene expression regulation (Refs 55, 155)	Mild behavioural and neurophysiological abnormalities (Ref. 53)	Allele-specific
SCA2	*ATXN2*	Possible role in RNA metabolism (Ref. 156)	Mild phenotype, including adult-onset obesity and fatty changes in liver (Ref. 157), some behavioural abnormalities (Ref. 158)	Non-allele-specific
SCA3	*ATXN3*	Deubiquitination (Ref. 57), transcriptional regulation (Ref. 159)	Mild behavioural changes (Ref. 58)	Allele-specific, non-allele-specific shows promise
SCA6	*CACNA1A*	Calcium channel controlling neurotransmitter release and calcium homeostasis (Ref. 8)	Severe phenotype including progressive ataxia, dystonia and altered neurotransmission (Ref. 54)	Allele-specific
SCA7	*ATXN7*	Component of TFTC/STAGA transcriptional coactivator complexes (Ref. 160), regulates retinal gene expression (Ref. 25)	Unknown	Allele-specific
SCA17	*TBP*	Initiation of transcription (Refs 161, 162)	Unknown	Gene knockdown and replacement

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Similar observations have been made in SCA1 and SCA6, in which knockout mice display severe neurological and behavioural abnormalities (Refs 53, 54). The proposed neuroprotective effect of wild-type *ATXN1* (in SCA1) (Refs 55, 56) and the lack of a pathological phenotype in SCA6 heterozygous null mice (Ref. 54) further motivate the use of allele-specific approaches for these diseases.

Wild-type *ATXN3* has also been shown to have a neuroprotective function (Ref. 57), although the mild behavioural changes observed in *ATXN3* knockout mice (Ref. 58) suggest that allele-specific silencing, while preferable, might not be essential for SCA3. A non-allele-specific approach might also be feasible in SCA2 and DRPLA, in which a loss of wild-type protein expression appears to be ameliorated by the presence of redundant proteins: ATXN2-related protein 1 (Ref. 59) and the short form of atrophin 2 (Atr2S) (Ref. 60), respectively.

The absence of a knockout model for SCA7 makes it difficult to determine the effect of a loss of wild-type function. However, the fact that *ATXN7* has an important role in the regulation of retinal gene expression (Refs 9, 10, 25) has prompted the suggestion that an allele-specific approach be adopted until further information becomes available.

The problem of haploinsufficiency in SCA17, as illustrated by cognitive deficits in mice heterozygous null for the TATA-binding protein (TBP) (Ref. 61), suggests that a loss of wild-type function in this disease would not be well tolerated. Thus, gene knockdown and replacement might represent the best strategy for treatment.

Finally, mice with a knockout mutation in the androgen receptor gene (*AR*) on the X-chromosome, responsible for SBMA, show severe developmental delays, while both humans and mice lacking a functional copy of AR present with testicular feminisation (Refs 62, 63). This indicates the need to retain wild-type function, preferably through the use of gene knockdown and replacement.

Principles and mechanisms of gene silencing

Therapeutic strategies based on the principle of RNAi make use of the endogenous mammalian gene silencing pathway, in which primary microRNA (miRNA) hairpins, transcribed from noncoding regions of the genome, are processed sequentially by a series of enzyme complexes to yield short double-stranded RNAs (Fig. 3a) (Ref. 64). In the final step, the antisense or 'guide' strand of the duplex is loaded into the RNA-induced silencing complex (RISC), which, in the case of post-transcriptional silencing, directs either cleavage or translational suppression of the complementary target mRNA, depending on the accuracy of base pairing (Refs 65, 66).

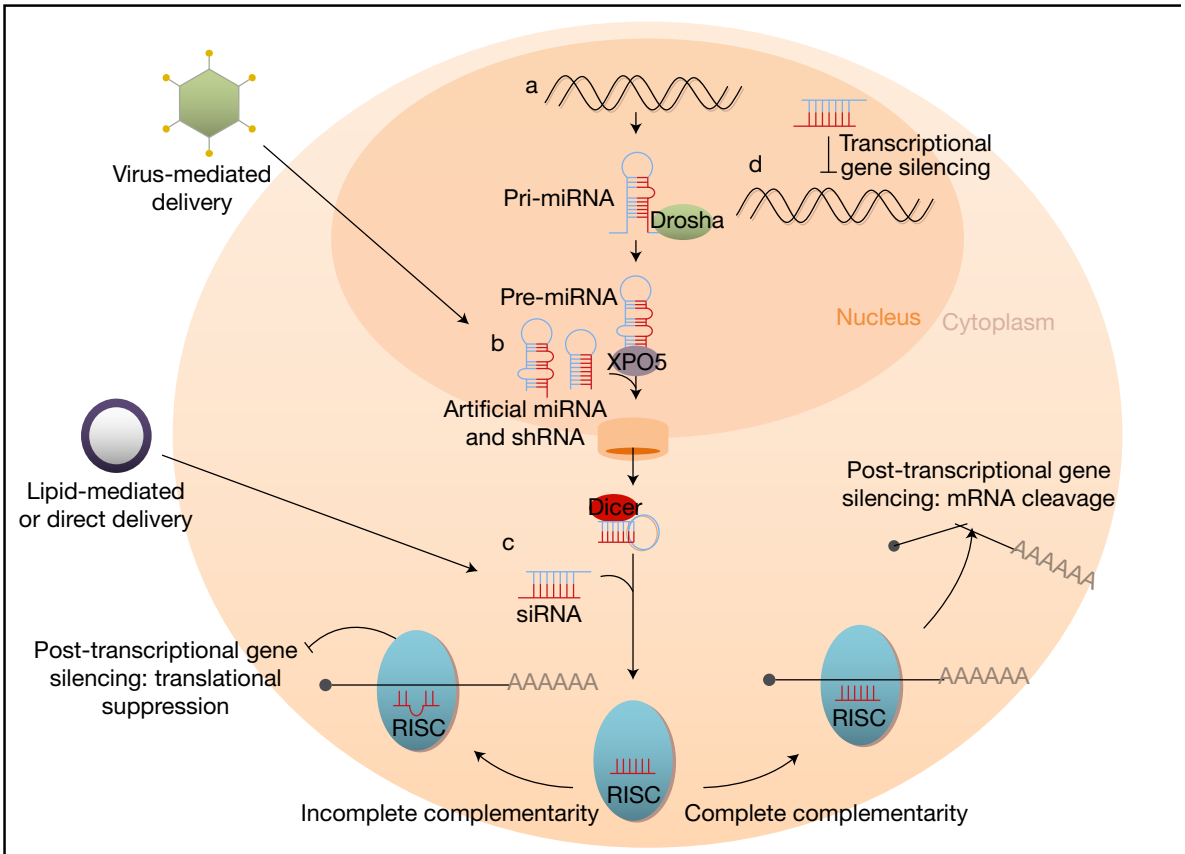
The pathway may be harnessed for therapeutic gene silencing through cytoplasmic delivery of synthetic short interfering RNAs (siRNAs), double-stranded molecules 21–22 nucleotides in length, which are designed to be perfectly complementary to the mRNA targeted for silencing (Fig. 3c) (Ref. 44). Short hairpin RNAs (shRNAs) represent a modification of this strategy in which the basic siRNA sequence is incorporated into a stem–loop construct, expressed at high levels from a PolIII promoter (Fig. 3b) (Ref. 67). However, because both siRNAs and shRNAs bind obligatorily to components of the RNAi pathway, large doses may result in saturation of the cellular RNAi processing machinery, disrupting endogenous miRNA biogenesis and function (Ref. 68). Additionally, the risk of off-target effects as a result of partial complementarity to unintended transcripts represents an important caveat to the clinical utility of RNAi, which is discussed in more detail below (Refs 69, 70).

One way of avoiding saturation involves the incorporation of synthetic siRNA sequences into artificial miRNAs under the control of a PolII promoter, allowing for regulated, cell-specific expression of the gene silencing molecules (Fig. 3b) (Ref. 69). The result is a decrease in accumulation of unprocessed precursors, leading to a reduction in cellular immune response and hence to an improvement in nonspecific, toxic effects (Refs 69, 71, 72).

Another method that has been proposed involves circumvention of the RNAi pathway altogether, through the use of AONs complementary to the mRNA targeted for silencing. AONs are short synthetic molecules, 15–25 nucleotides in length, which hybridise to their target mRNA by means of Watson and Crick base pairing (Ref. 73). Post-transcriptional gene silencing is achieved through RNAse H-mediated intranuclear degradation of the target transcript (Ref. 74) or steric blocking of translation (Ref. 75).

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RNA interference-based therapies make use of the endogenous mammalian gene silencing pathway

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Figure 3. RNA interference-based therapies make use of the endogenous mammalian gene silencing pathway. (a) Endogenous primary microRNAs (pri-miRNAs), transcribed from genomic DNA, are processed in the nucleus by Drosha (an RNase III enzyme) and its interaction partners to yield pre-miRNAs. These are exported to the cytoplasm by means of exportin-5 (XPO5), where they are recognised and cleaved by the Dicer enzyme complex, resulting in the production of ~22 bp staggered duplexes. The guide strand (depicted in red) is loaded into the RNA-induced silencing complex (RISC), which, in the case of post-transcriptional silencing, directs cleavage or translational suppression of the cognate mRNA, depending on the accuracy of base pairing between the guide strand and the target mRNA (Ref. 66). (b) The pathway may be manipulated for therapeutic gene silencing through the introduction of artificial miRNA or short hairpin RNA (shRNA) constructs complementary to the gene or allele targeted for repression. These effectors are expressed under the control of PolII and PolIII promoters, respectively, and enter the pathway upstream of Dicer processing. (c) Alternatively, short interfering RNAs (siRNAs) that target the gene of interest can be incorporated directly into RISC. (d) Long-term suppression of gene expression may be accomplished through TGS, in which siRNAs complementary to the promoter region of the target gene induce epigenetic changes. Delivery is usually mediated by integrating lenti- or episomally-expressing (rAAV) viruses (in the case of shRNAs and miRNAs) or by direct injection or lipid-mediated transfection (in the case of siRNAs).

Non-allele-specific gene silencing

Numerous studies involving therapeutic gene silencing for polyQ diseases have been undertaken over the past decade (Ref. 38), many of which have adopted the less technically challenging non-allele-specific approach. A significant number of these use transgenic models, in which an exogenous mutant gene or gene fragment is introduced into a model system in which two normal endogenous copies

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of the gene of interest are already present (Ref. 76). In these cases, silencing efficacy is evaluated based on the ability of the effector to selectively target the transgene sequence, rather than its ability to distinguish between endogenous pathogenic and normal alleles (Ref. 77). Nevertheless, such studies have provided valuable evidence for the feasibility of gene silencing as a therapeutic approach.

In *Drosophila* and mammalian cell models of SBMA, for instance, sequence-specific inhibition of a truncated green fluorescent protein (GFP)-conjugated *AR* gene through the use of short double-stranded RNA resulted in decreased expression and aggregation of the mutant protein, ultimately rescuing toxicity and caspase-3 activation (Ref. 78). Similar results have been achieved in models of SCA1 and HD, using a variety of RNAi (Refs 77, 79) and AON (Refs 39, 80)-based approaches.

These investigations have also enabled the development of sophisticated and effective delivery systems, supporting the development of RNA-based silencing methods for HD therapy in particular. One such example is the use of recombinant adeno-associated viral vectors capable of sustained suppression of mutant *HTT* expression both in vitro and in vivo, which have been shown to partially ameliorate both cellular and behavioural manifestations of HD in R6/1 and HD-N171-82Q transgenic mice (Refs 77, 81). These findings emphasise the effectiveness of RNAi, as both neuroprotective (Ref. 82) and capable of reversing neuropathology (Ref. 83). Such viral delivery systems represent a significant improvement over delivered siRNAs and AONs in terms of duration of treatment, but present new challenges with regard to dosage regulation, which are discussed in more detail below.

It is important to note, however, that suppression of a transgene against the background of sustained endogenous gene expression provides little information about the effect of non-allele-specific silencing on the wild-type copy of the gene and hence, of the potential need for allele-specific approaches. This, together with the technical challenges associated with allele-specific silencing, has promoted renewed interest in non-allele-specific silencing of endogenous genes, to determine the in vivo effects of suppression of wild-type function (Ref. 76).

Although a loss of function of wild-type HTT protein has previously been shown to be deleterious, two recent studies have succeeded in silencing both mutant and wild-type *HTT* in transgenic mouse models of HD with minimal phenotypic effects (Refs 37, 76). Boudreau and colleagues demonstrated that a 75% reduction in both mutant and endogenous wild-type *HTT*, mediated by miRNA-based RNAi expression vectors, improved motor coordination and survival in HD-N171-82Q mice, although some weight loss was observed relative to the untreated group (Ref. 76). *HTT* silencing did, however, result in transcriptional alterations of more than 400 genes, corresponding to previously established functions of *HTT*. Several of these transcripts have been shown to be similarly dysregulated in transgenic HD models, supporting the concept of a loss-of function component of HD (Ref. 76). Using a lentiviral-vector-expressed shRNA, Drouet et al. (Ref. 37) showed that silencing of both mutant and endogenous wild-type HTT to 25–30% in vivo did not induce toxicity or striatal vulnerability in HD171-82Q rats, which overexpress a pathologically expanded N-terminal fragment of human huntingtin protein. Indeed, administration of the shRNA constructs resulted in marked improvement in a number of cellular markers of disease (including reduced aggregate formation and restoration of striatal gene expression), even in cases where treatment was initiated after the onset of disease symptoms, confirming earlier findings regarding the reversible nature of polyQ-induced striatal pathology. Despite the appearance of similar transcriptomic changes to those observed by Boudreau et al. (Ref. 76), the fact that partial silencing of both transcripts appears to be well tolerated for up to 9 months post-treatment suggests that partial loss of wild-type function does not exacerbate HD pathology (Ref. 37).

Recent findings suggest that SCA3 may also be amenable to non-allele-specific silencing (Ref. 84). A previous study in *Drosophila*, demonstrating that loss of wild-type *ATXN3* function contributes to SCA3 pathology, led to a focus on allele-specific therapies in order to retain the reportedly neuroprotective wild-type protein (Ref. 57). However, a subsequent investigation into the effects of shRNA-mediated silencing of

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endogenous *ATXN3* in wild-type and SCA3 rat models suggests strongly that global *ATXN3* silencing does not aggravate disease symptoms, but in fact reduces neuropathology, resulting in an 80% decline in ATXN3-positive inclusions (Ref. 84).

It is possible that a threshold level of wild-type expression is required to avoid deleterious effects in such cases, although determining this level for human disease may prove challenging. In addition, tolerance of wild-type gene silencing may differ between species, depending on the degree of redundancy of protein function. Such results are nevertheless significant because they demonstrate the feasibility of non-allele-specific silencing approaches, which are not only simpler to develop, but offer the possibility of treating vast numbers of patients rather than a select few who meet the criteria for allele-specific techniques. Allele specificity is, however, still vital in those cases where maintenance of wild-type protein expression is essential for cellular function.

Allele-specific gene silencing

Allele-specific silencing is accomplished through the exploitation of differences in nucleotide sequence between wild-type and mutant alleles, making it particularly applicable to dominantly inherited disorders such as polyQ diseases, in which only one copy of the mutant gene is required for the disease to manifest. The aim is thus to achieve maximum selectivity for the mutant transcript, while ensuring that the wild-type allele is spared the effects of gene silencing (Ref. 44). Because the sequence of the effector must match the target exactly to elicit RISC- or RNase H-mediated degradation, it is vital that the mutant and wild-type transcripts of the gene to be targeted differ by at least one nucleotide, in order to facilitate discrimination. In many diseases caused by point mutations, insertions or deletions, the targetable sequence difference is offered by the mutation itself (Refs 85, 86, 87). However, in the case of polyQ neurodegenerative diseases, targeting the CAG expansion presents a challenge because the polymorphic nature and ubiquitous presence of the repeat tract throughout the genome make it difficult to target specifically, particularly because effectors <25 nucleotides in length are unlikely to be able to distinguish between wild-type and pathologically expanded CAG tracts (Ref. 45).

Most efforts have therefore focused on targeting sequence differences created by the presence of SNPs linked to the CAG repeat expansion in a specific patient group (Ref. 44), using RNAi-based technologies. Once a suitable SNP has been identified, several additional mechanistic issues must be taken into account when designing siRNAs capable of optimal discrimination between transcripts that differ at a single nucleotide (Ref. 88). Of critical importance, the allele of the SNP linked to the mutant copy of the gene should be determined in order to determine the correct siRNA sequence required. This may be accomplished through studies of conserved haplotypes within patient cohorts or through more direct methods such as allele-specific reverse trancriptase PCR (Ref. 89). Where possible, purine:purine mismatches should be selected as targets, because these result in the greatest disruption of the siRNA–mRNA helix, and hence confer the greatest degree of discriminatory power (Ref. 90). In cases where purine:purine mismatches are unavailable, the introduction of additional mismatches, particularly in the seed region of the siRNA, which directs binding and recognition of the target mRNA, may improve the selectivity of SNP sites predicted to be poor candidates for the development of allele-specific siRNAs (Ref. 91). In general however, mismatches in the 5′ seed region offer only moderate discrimination, whereas mismatches 3′ to the seed region, in particular at positions 10 and 16 of the siRNA, promote robust allele selectivity (Ref. 92). This is probably due to the functions of the different regions, as allele specificity is enhanced when the conformation of the siRNA-target complex blocks catalysis and RISC-mediated cleavage rather than siRNA binding (Ref. 88). In addition to design, the concentration of effector administered is also important, because allele specificity has been shown to be concentration dependent (Ref. 93). The most successful allele-specific treatment is therefore likely to be that for which adequate discrimination is achieved at a concentration sufficient to effect a clinically significant level of mutant protein inhibition (Refs 88, 92).

In the case of SCA3, a C to G SNP at position 987 in *ATXN3*, which cosegregates with the

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disease, has formed the basis of a successful allele-specific silencing approach (Ref. 94). Because ~70% of disease alleles carry a 'C' at this site (Ref. 95), RNAi-based effectors have been developed to selectively silence the mutant copy of the gene in vitro, based on sequence complementarity to the region of the transcript including the C-allele of the SNP (Ref. 96). Efficient and selective silencing of mutant *ATXN3* has since been demonstrated in vivo, using lentiviral vectors encoding siRNAs targeting the same SNP to achieve significant reductions in mutant gene expression, aggregate formation and neurodegeneration in a rat model of SCA3 (Ref. 97). A similar approach has been used to achieve allele-specific silencing of mutant *ATXN7* in HEK293 cells, targeting the A-allele of a SNP in linkage disequilibrium with the pathogenic expansion, the result of a founder effect in South African SCA7 patients (Ref. 98).

Although knockdown of wild-type *HTT* has recently been shown to be tolerated in vivo (Ref. 84), the important role of HTT in the development and maintenance of the CNS suggests that allele-specific approaches might still be preferable for HD (Ref. 99). To this end, recent investigations have examined the feasibility of targeting disease-linked polymorphisms in patient fibroblasts. Following the success of allele-specific *HTT* silencing in a HeLa cell model (Ref. 88), an siRNA was designed to specifically target the mutant *HTT* transcript in a patient heterozygous for the rs363125 SNP, in which the C-allele was found to be linked to the expansion (Ref. 89). Transfection of cultured patient fibroblasts with the siRNA resulted in an ~80% reduction in mutant mRNA and protein expression, whereas wild-type levels were not significantly altered (Ref. 89). A similar study (Ref. 93) evaluated the efficacy of allele-specific siRNAs in patient fibroblasts carrying the three-base-pair Δ2642 deletion, present in 38% of mutant HD, but only 7% of wild-type HD alleles (Ref. 52). SiRNAs designed to target the deletion reduced expression of mutant *HTT* by up to 50% in patient fibroblasts, and showed similar potent, dose-dependent effects in HeLa- and SH-SY5Y-based luciferase reporter systems, suggesting that RNAi-based therapy is likely to be effective in a range of different cell types. Allele-specific knockdown of the mutant allele also resulted in partial correction of HD-associated nuclear abnormalities in patient fibroblasts, while avoiding the activation of caspase-3 associated with silencing of both copies of the gene (Ref. 93).

These results represent an important improvement on previous studies, because they demonstrate the potential of RNAi to successfully discriminate between endogenous single-nucleotide mismatches present in patient DNA rather than relying on sequence differences between species to selectively silence mutant *HTT*. However, it should be noted that the cost and effort required to develop large numbers of effectors for low-frequency disease-linked SNPs are likely to hinder the progress of allele-specific therapy towards clinical trials (Refs 89, 93).

To address this issue, large-scale studies of polymorphic sites within the *HTT* gene have been conducted in the European and North American HD patient populations (Refs 90, 92). The finding that as few as five allele-specific siRNAs, targeting three SNP sites, could be used to treat 75% of these patients should greatly simplify the development of therapy (Ref. 90). Including a further four SNPs increases the proportion of eligible patients to 85.6%, although targeting these additional sites would require the design of a significantly greater number of siRNAs (Refs 90, 92). It is difficult to extrapolate these results from the European Caucasian patient sample to other patient populations around the world. However, a similar investigation into targetable polymorphisms in Venezuelan HD patients has revealed six SNPs, which would provide options for allele-specific silencing for up to 63.5% of the patient population (Ref. 92). Thus, it may be theoretically possible to develop a relatively small number of effectors to deliver effective allele-specific RNAi therapy to the majority of polyQ disease patients.

Recent developments in AON technology offer the opportunity to develop therapies applicable to all patients across the polyQ disease spectrum, through the discovery of molecules capable of allele-specific silencing based solely on differences in CAG repeat length (Refs 75, 100). Discrimination is thought to be based on differences in secondary structure or number of binding sites, depending on the size of the CAG repeat tract, with larger repeats demonstrating increased susceptibility to AON binding

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(Refs 75, 100). The result is steric blocking of translation, rather than RNase H-mediated cleavage of the target mRNA (Ref. 75).

Among the single-stranded antisense molecules investigated as potential effectors, peptide nucleic acids (PNAs) and locked nucleic acids (LNAs) demonstrate particularly robust and allele-specific silencing of mutant *HTT* and *ATXN3* alleles, exhibiting up to eightfold selectivity (Ref. 75), with relatively low levels of toxicity (Ref. 100) in patient fibroblasts and cell models of disease. The use of AONs, as opposed to RNAi-based mechanisms, is particularly appealing for a number of reasons: single-stranded molecules are cheaper and simpler to synthesise and may be more efficiently distributed in vivo because of their smaller size (Ref. 75). In addition, bypassing the cellular gene silencing complexes should result in more predictable off-target effects, while allowing for more extensive chemical modifications such as the introduction of pharmacologically favourable phosphorothioate linkages, which improve biodistribution and increase stability (Ref. 74). Of note, several AON-based therapeutic approaches are currently being explored in clinical trials, paving the way for use of these technologies in the treatment of polyQ diseases (Refs 101, 102).

Allele-specific targeting of the CAG repeats is not limited to AONs, however. A recent report (Ref. 103) suggests that artificial miRNAs (mismatch-containing duplexes complementary to the CAG expansion) might offer a potent and versatile silencing mechanism, capable of >31-fold selectivity in vitro despite the introduction of up to four mismatched bases into the guide strand.

There are still some concerns regarding the use of CAG-targeting AONs, which might limit their efficacy in vivo. In particular, the presence of CAG tracts throughout the genome suggests the potential for deleterious off-target effects, although no silencing of repeat-containing genes (including *TBP*, *ATN1* and *FOXP2*) has yet been observed in vitro (Refs 75, 103). The fact that more common smaller CAG expansions appear to be less amenable to silencing using this approach, compared with rarer large expansions, also presents a challenge to the clinical utility of this method, which must be addressed before such approaches can progress to clinical trials (Ref. 75).

Gene knockdown and replacement

One alternative to sequence-based allele-specific silencing, which still results in retention of wild-type protein function, is the gene knockdown and replacement method. Silencing of both mutant and wild-type alleles of the disease gene is accomplished through the use of non-allele-specific RNAi. This is accompanied by the introduction of a full-length, codon-optimised 'replacement' copy of the wild-type gene, whose nucleotide sequence is altered in such a way as to retain the amino acid sequence while abolishing the siRNA recognition site (Ref. 104).

In SCA6, the CAG repeat length of mutant alleles falls within the normal range of other polyQ diseases, making allele-specific approaches based on repeat-length targeting inappropriate. In a cell model of SCA6, transfection of an siRNA capable of silencing both the mutant and wild-type *CACNA1A*, together with an exogenous copy of the gene that is resistant to the gene silencing effector, resulted in restoration of wild-type protein function (Ref. 47). A similar approach has subsequently been tested in vivo, to restore wild-type SOD1 function in a mouse model of amyotrophic lateral sclerosis (ALS) (Ref. 46).

This method has the distinct advantage of being able to circumvent the laborious process of identification and validation of allele-specific siRNAs targeting disease-linked polymorphisms, particularly in diseases where no single sequence change can be targeted in a large number of patients (Ref. 46). However, the expression of the restored wild-type protein would need to be tightly regulated, to prevent the development of side effects. In addition, delivery methods need to be optimised to ensure that every cell treated receives both the RNAi effector and the modified replacement gene, suggesting that this method may not be as straightforward as originally believed (Ref. 47).

Novel gene silencing approaches

Transcriptional gene silencing

Although most work on gene silencing therapies for polyQ diseases has been directed at the post-transcriptional level, TGS has also been proposed as a method to specifically and permanently suppress disease gene expression, avoiding the need for continuous delivery of RNAi- or AON-based effectors (Fig. 3d). TGS is

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based on an endogenous miRNA-mediated silencing mechanism first observed in plants (Ref. 105), and involves the design of artificial miRNAs to target promoter regions of the gene, resulting in transcriptional suppression associated with DNA methylation and histone deacetylation (Refs 106, 107, 108). The precise pathways underlying mammalian TGS have yet to be fully elucidated (Refs 64, 107), although evidence suggests a requirement for endogenous promoter-associated RNA in order to direct chromatin modifications (Ref. 109). It is unclear whether such an approach could be adapted to achieve allele specificity, and at what stage of disease the therapy may need to be administered. Nonetheless, it is likely that such techniques may offer a means for long-term suppression of disease genes in time.

Targeting toxic RNA species

PolyQ diseases have traditionally been thought to result solely from toxic gain of function of the mutant protein. However, the identification of additional pathogenic mechanisms, such as RNA toxicity in SCA3 (Ref. 110), offers the opportunity to develop novel therapies capable of disrupting aberrant RNA secondary structure (Ref. 111) or protein sequestration (Ref. 112) that may result from the expansion of a CAG repeat (Refs 113, 114). A recent study demonstrating the ability of phosphorodiamidate morpholino AONs to block RNA–protein interactions responsible for the development of myotonic dystrophy, through direct binding to the expanded CUG repeat, suggests that similar methods may be adopted to modulate the effects of toxic RNA in CAG repeat diseases (Ref. 112).

miRNAs as therapeutic targets

In addition to the use of miRNA mimics as therapeutic effectors, several lines of evidence support the role of endogenously expressed miRNAs themselves in the process of neurodegeneration and highlight their role as potential therapeutic targets (Ref. 115). Patterns of differential miRNA expression have been identified in several polyQ diseases, including HD (Ref. 116) and SCA1 (Ref. 117), as well as in other neurodegenerative diseases (reviewed in Ref. 118). In addition, the identification of naturally occurring miRNAs that modulate the expression and toxicity of polyQ disease proteins in vivo (Refs 115, 117) might guide the development of therapeutic effectors for future studies. In particular, the discovery that the miRNA *bantam* (*ban*) mitigates the pathogenicity of polyQ-expanded *ATXN3* in *Drosophila* by targeting the apoptosis pathway suggests the possibility of common miRNA-based therapies for polyQ diseases, targeting cell survival pathways rather than specific disease-causing genes (Ref. 119). The central role of miRNAs in neurodegeneration is further underscored by finding, in several model organisms, that *Dicer* deficiency, characterised by a loss of mature miRNAs, leads progressively to degeneration of Purkinje cells, cerebellar degeneration and ataxia (Refs 120, 121).

Among the novel miRNA-based therapeutics currently being developed are anti-miRNAs (molecules designed to downregulate endogenous miRNA expression), which may be used to suppress apoptotic pathways and augment natural cell survival mechanisms (Ref. 122).

Numerous chemically modified 'anti-miRs' or 'antago-miRs' have been tested for efficacy and potency both in vitro and in vivo (Ref. 123). These include LNA-modified oligonucleotides, which demonstrate enhanced specificity at low doses, are well tolerated in vivo (Ref. 124), and are capable of both hindering miRNA-target binding and decreasing miRNA levels (Refs 125, 126). LNA-modified anti-miRNAs may be further enhanced through the incorporation of an endonucleocytic DNAzyme motif, facilitating cleavage and hence more rapid clearance of the target miRNA (Ref. 127). More extensive testing is necessary in order to assess the dosage, activity and clearance of such molecules in in vivo models of neurodegeneration. However, data from recent phase I and II trials using LNA-based anti-miRNAs to mitigate hepatitis C replication should provide valuable insights into the clinical application of these technologies (Ref. 128).

Clinical considerations

The success of several preclinical and proof-of-principle studies has clearly demonstrated the potential of RNA-based therapeutics for the treatment of polyQ disease. However, numerous issues regarding safety, stability and specificity must be addressed before such approaches can be transitioned into the clinical setting.

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Off-target effects

Of major concern is the ability of RNAi and AON effectors to result in nonspecific gene silencing. Three broad categories of off-target effects are commonly observed: unintended seed pairing to transcripts with similar sequences to the target mRNA (Ref. 69); immune stimulation, through activation of the interferon-mediated innate immune response (Refs 129, 130); or saturation of the endogenous gene silencing machinery, with widespread cellular effects (Ref. 131).

Off-target effects caused by nonspecific target binding arise from regions of partial sequence complementarity between siRNAs and mRNAs, as short as eight base pairs in length (Ref. 132). The result is a moderate (less than twofold) downregulation of the off-target transcripts, reminiscent of the effects of endogenous miRNAs on gene expression, giving rise to the term 'miRNA-like off-target effects' (Ref. 133). Although off-target activity has been shown to be elevated at high doses of siRNA (Ref. 134), decreasing the dosage alone does not rescue cells from the phenotypic effects of erroneous gene silencing (Ref. 133). Numerous approaches have therefore been suggested to mitigate miRNA-like off-target effects, including the use of prediction algorithms (Ref. 135), and siRNA pooling, in which competition between multiple siRNAs for the same target leads to a selective reduction of off-target effects, because of their unique off-target signatures (Refs 131, 136). Of greater clinical relevance is the incorporation of chemical modifications, such as unlocked nucleic acids or 2′-*O*-methyl modifications, into the seed region of synthetic siRNAs during manufacture (Refs 137, 138, 139). The introduction of a 2′-*O*-methyl modification at position 2 of the siRNA guide strand has been demonstrated to reduce silencing of ~80% of off-target transcripts by an average of ~66% (Ref. 139). Although its effects have not yet been evaluated in vivo, these results suggest that introduction of a 2′-*O*-methyl modification at position 2 of the siRNA seed region is likely to enhance the specificity of therapeutic siRNAs (Ref. 139).

Stimulation of interferon responses, which result in global downregulation of gene expression (Ref. 38), may be caused by the siRNAs themselves (nucleotide sequence or structure) or by the vehicles used to deliver them in vivo (Ref. 131). The expression of proinflammatory cytokines is triggered by sequence-specific recognition of siRNAs, mediated by Toll-like receptors (TLRs) 3, 7 and 8, and may thus be prevented by avoiding immunostimulatory sequences during effector design (Ref. 140) or by incorporating 2′ modifications to the ribose backbone, which block TLR recognition (Refs 131, 141). Switching from si- or shRNAs to lower-expressing artificial miRNAs has also been shown to improve the immunogenic profile of RNA-based therapies (Refs 69, 71). Because TLRs 3, 7 and 8 are present in endosomes, delivery vehicles such as cationic liposomes, which make use of the endosomal pathway, are more likely to activate an immune response (Ref. 142). The use of alternative delivery mechanisms such as electroporation should eliminate these effects, except in cases where TLR3 is expressed as a cell-surface marker, such as the retinal endothelium (Ref. 143).

The global perturbation of gene expression caused by si- and shRNA-mediated saturation of the RNAi machinery is an important clinical consideration. Exogenous silencing effectors compete with cellular miRNAs for components of the RNAi pathway such as RISC or exportin 5, disrupting endogenous miRNA biogenesis and function (Refs 69, 131, 144). Artificial miRNAs, which closely mimic the structure and expression levels of their endogenous counterparts, have been shown to avoid this interference and are well tolerated both in vitro and in vivo (Ref. 69). Mitigation of RNAi toxicity through the use of such constructs in mouse models of both HD (Ref. 72) and SCA1 (Ref. 69) suggests that artificial miRNAs may offer a more appropriate means of expressing inhibitory RNAs in the CNS.

Dosage and delivery

Saturation of the endogenous gene silencing complex occurs at high doses of RNAi (Ref. 68), whereas low doses reduce the efficiency of transduction, and might diminish the effectiveness of silencing throughout larger brain regions in vivo (Ref. 38); thus, dosage titration is another important consideration for therapy. Changes to the therapeutic dose can be accomplished by manipulating the amount and stability of vector delivered, the stability and efficiency of RNAi transport and processing, or the strength of the promoter (Ref. 76). These are

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important considerations when choosing a silencing approach: integrated (lentiviral) or episomal (AAV) viral vectors are capable of sustained, long-term expression, whereas delivered siRNAs and AONs, although transient in their effects, have the advantage of allowing rapid discontinuation of expression in the event of toxicity. PolII-expressed artificial miRNAs represent an attractive compromise, offering the potential to lower the cellular expression of the effector without reducing the viral dose administered to cells (Ref. 38).

The duration of silencing varies from 2 to 4 weeks (siRNAs) to several months (AONs), and depends largely on the stability of the effector, as well as on the rate of cell division, the half-life of the protein and the extent of expression of the target gene (Ref. 102). Because AONs are not dependent on recognition by components of the endogenous gene silencing pathway to mediate their effects, they are more amenable to backbone modifications than siRNAs. Indeed, the incorporation of PNAs, LNAs and other modified nucleic acids has been shown to enhance AON silencing through improved stability and resistance to endonuclease degradation (Ref. 75).

Perhaps the most important consideration, however, is the issue of optimised delivery. Although much success has been achieved in vitro using cationic lipid-mediated transfection (Ref. 145), there is evidence to suggest that these reagents may be toxic in vivo, necessitating the development of more sophisticated methods for use in clinical trials. Therapies targeting components of the CNS face the added challenge of crossing the blood–brain barrier (Refs 38, 145). This may be overcome through the use of viral vectors capable of transducing postmitotic neurons (Refs 146, 147), the efficacy of which has already been demonstrated in preclinical trials (Ref. 148). Strategies that make use of specific receptors [such as the use of rabies virus glycoprotein to target siRNA to neurons expressing nicotinic acetylcholine receptors (Ref. 149), or nanoparticles (Ref. 150)] have also proved successful in preclinical studies and might hold promise for the delivery of gene silencing therapy to the CNS in humans. An additional consideration regarding delivery is the need to specifically and directly target affected areas of the brain, which could be accomplished through direct intrathecal or intraparenchymal administration of the therapeutics to affected regions (Refs 102, 151). A promising development in this regard is the recent announcement by the CHDI Foundation of its collaboration with two companies – Medtronic and Alnylam – to develop an implantable infusion system capable of delivering anti-HTT siRNAs directly into the brains of HD patients (Ref. 145).

Clinical applications

There are currently 14 active clinical trials involving RNAi for a range of conditions including age-related macular degeneration and cancers, although none involves the CNS (Ref. 152). The studies make use of a wide variety of delivery approaches, from direct local injection of unmodified siRNAs to viral-mediated and systemic delivery. Preliminary data suggest mixed success, with a number of trials progressing through Phase I with minimal safety concerns and modest silencing efficacy, and some even showing potential for allele specificity. A small number of studies, aimed at developing treatment for wet age-related macular degeneration, have been discontinued, owing to concerns regarding the effectiveness of treatment (Ref. 152).

AONs are currently being tested in a number of trials for conditions such as Duchenne muscular dystrophy and several cancers (www.clinicaltrials.gov). The results of an AON clinical study targeting apolipoprotein B have shown persistent gene silencing for up to 3 months after cessation of treatment: a reminder of the need to monitor the half life of silencing molecules, in order to be able to rapidly terminate drug exposure, should the need arise (Ref. 101). The first clinical study of AONs in neurodegenerative disease (ALS) has recently been initiated (Ref. 102), and should provide valuable insights over the coming years into the optimum parameters required to safely and effectively silence disease-causing genes in the human CNS.

Research in progress and outstanding research questions

RNAi and AONs provide novel and promising approaches for the treatment of polyQ diseases, offering the potential to attenuate pathology through direct intervention at the mRNA level to suppress production of the disease-causing

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protein (Table 2). Preclinical studies have demonstrated the ability of both approaches to direct potent, sequence-specific silencing of a range of polyQ mutant proteins in vivo, resulting in amelioration of both neurotoxicity and behavioural abnormalities.

Allele-specific silencing is particularly relevant to diseases in which a loss of wild-type protein function is unlikely to be tolerated. Knockout data from SCA1 and SCA7 studies suggest the contribution of a loss of function to pathogenesis, which may necessitate the development of allele-specific approaches (Refs 53, 54). Although gene knockdown and replacement therapy have been tested in SCA6 models in vitro, the necessity of retaining at least one functional *CACNA1A* suggests that SCA6, too, may be amenable to allele-specific silencing (Ref. 54).

Practical considerations for allele-specific silencing targeting either SNPs linked to the mutant allele, or the CAG repeat itself, include selectivity for the target mRNA over other transcripts, degree of discrimination between wild-type and mutant alleles, dosage requirements for specific and selective silencing in vivo, and the number of target SNPs required to deliver allele-specific silencing to the majority of patients (Ref. 102). The recent discovery that a small number of polymorphisms may provide therapeutic targets for more than three quarters of the European HD patient population suggests that the design of SNP-based allele-specific RNAi may not be as laborious as was once thought (Ref. 92). It remains to be seen, however, whether such results can be replicated for other polyQ diseases, although the availability of high-throughput sequencing technologies and vast amounts of SNP data through the HAPMAP project (http://hapmap.ncbi.nlm.nih.gov/) is likely to simplify the identification of discriminating SNPs in the future (Ref. 45).

A key issue that remains to be addressed concerns the recent success of non-allele-specific silencing in HD and SCA3 – diseases in which knockout models have shown some evidence of a deleterious phenotype (Refs 49, 58). The results suggest that partial loss of wild-type polyQ proteins may be tolerated in vivo with minimal phenotypic consequences, raising the

Table 2. Comparison of RNAi- and AON-based gene silencing effectors

	siRNA	shRNA/artificial miRNA	AONs
Delivery method	Viral and nonviral	Viral and nonviral	Nonviral
Control over dosage	Direct	Indirect (promoter dependent)	Direct
Cellular site of action	Cytoplasm	Nucleus/cytoplasm	Nucleus/cytoplasm
Strategy for allele specificity	SNP targeting (Refs 88, 89)	CAG targeting (Ref. 103), potential for SNP targeting (Ref. 98)	CAG targeting (Ref. 75)
Saturation of RNAi pathway	Yes (Ref. 68)	Minimal (Ref. 69)	No
Duration of silencing	2–4 weeks (Ref. 163)	Not established	>3 months (Ref. 101)
Immunogenicity	Avoided by modification (Ref. 140)	Avoided by adopting the miRNA mechanism (Ref. 71)	Avoided by modification (Ref. 164)
Clinical trials	Multiple trials (non-CNS) (Ref. 152)	None	Multiple trials (non-CNS) (Ref. 165), 1 CNS trial (ALS) (Ref. 102)

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possibility of bypassing the technical challenges of allele-specific silencing in other diseases, in favour of the more straightforward total knockdown approach. To evaluate the prospect of non-allele-specific silencing in other polyQ diseases, a number of questions will need to be answered, pertaining to the threshold level of wild-type protein expression required to maintain cellular function, the presence of functionally redundant proteins, and the availability of human 'models' of loss of function of the disease gene. Improved animal models that more closely recapitulate the disease phenotype should aid in addressing at least some of these questions.

Optimising delivery methods and choice of effectors are likely to be the major considerations as gene silencing therapies for polyQ diseases progress towards clinical trials. However, thorough preclinical investigations that evaluate both specificity and selectivity in vivo, together with the results of upcoming RNAi- and AON-based clinical studies, should provide valuable data on the safest and most efficacious approaches to silencing.

Recent advances in the field of RNA-based therapeutics have led to the development of sophisticated mechanisms of silencing with vastly improved toxicity profiles. As RNAi- and AON-based gene silencing becomes increasingly feasible, it is likely that effective treatments for polyQ diseases will progress rapidly towards clinical application.

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References

1 Durr, A. (2010) Autosomal dominant cerebellar ataxias: polyglutamine expansions and beyond. Lancet Neurology 9, 885-894
2 Stevanin, G. et al. (1998) *De novo* expansion of intermediate alleles in spinocerebellar ataxia 7. Human Molecular Genetics 7, 1809-1813
3 Garden, G.A. and La Spada, A.R. (2007) Molecular pathogenesis and cellular pathology of spinocerebellar ataxia type 7 neurodegeneration. Cerebellum 99999, 1-12
4 Globas, C. et al. (2008) Early symptoms in spinocerebellar ataxia type 1, 2, 3, and 6. Movement Disorders 23, 2232-2238
5 Lebre, A.S. and Brice, A. (2003) Spinocerebellar ataxia 7 (SCA7). Cytogenetic Genome Research 100, 154-163
6 Pulst, S.M. et al. (2005) Spinocerebellar ataxia type 2: polyQ repeat variation in the CACNA1A calcium channel modifies age of onset. Brain 128, 2297-2303
7 Li, S.H. and Li, X.J. (2004) Huntingtin-protein interactions and the pathogenesis of Huntington's disease. Trends in Genetics 20, 146-154
8 Saegusa, H. et al. (2007) Properties of human Cav2. 1 channel with a spinocerebellar ataxia type 6 mutation expressed in Purkinje cells. Molecular and Cellular Neuroscience 34, 261-270
9 La Spada, A.R. et al. (2001) Polyglutamine-expanded ataxin-7 antagonizes CRX function and induces cone-rod dystrophy in a mouse model of SCA7. Neuron 31, 913-927
10 Helmlinger, D. et al. (2006) Glutamine-expanded ataxin-7 alters TFTC/STAGA recruitment and chromatin structure leading to photoreceptor dysfunction. PLoS Biology 4, 432-445
11 Yoo, S.Y. et al. (2003) SCA7 knockin mice model human SCA7 and reveal gradual accumulation of mutant ataxin-7 in neurons and abnormalities in short-term plasticity. Neuron 37, 383-401
12 Bichelmeier, U. et al. (2007) Nuclear localization of ataxin-3 is required for the manifestation of symptoms in SCA3: in vivo evidence. Journal of Neuroscience 27, 7418-7428
13 Burright, E.N. et al. (1995) SCA1 transgenic mice: a model for neurodegeneration caused by an expanded CAG trinucleotide repeat. Cell 82, 937-948
14 Goti, D. et al. (2004) A mutant ataxin-3 putative-cleavage fragment in brains of Machado-Joseph disease patients and transgenic mice is cytotoxic above a critical concentration. Journal of Neuroscience 24, 10266-10279

Accession information: doi:10.1017/erm.2011.1; Vol. 14; e3; January 2012
© Cambridge University Press 2012

https://doi.org/10.1017/erm.2011.1 Published online by Cambridge University Press

15 Watase, K. et al. (2002) A long CAG repeat in the mouse Sca1 locus replicates SCA1 features and reveals the impact of protein solubility on selective neurodegeneration. Neuron 34, 905-919
16 Huynh, D.P. et al. (2003) Expansion of the polyQ repeat in ataxin-2 alters its Golgi localization, disrupts the Golgi complex and causes cell death. Human Molecular Genetics 12, 1485-1496
17 Cemal, C.K. et al. (2002) YAC transgenic mice carrying pathological alleles of the MJD1 locus exhibit a mild and slowly progressive cerebellar deficit. Human Molecular Genetics 11, 1075-1094
18 Shirendeb, U. et al. (2011) Abnormal mitochondrial dynamics, mitochondrial loss and mutant huntingtin oligomers in Huntington's disease: implications for selective neuronal damage. Human Molecular Genetics 20, 1438-1455
19 Yamanaka, T. and Nukina, N. (2010) Transcription factor sequestration by polyglutamine proteins. Methods in Molecular Biology (Clifton, NJ) 648, 215-229
20 Sugars, K.L. and Rubinsztein, D.C. (2003) Transcriptional abnormalities in Huntington disease. Trends in Genetics 19, 233-238
21 Einum, D.D. et al. (2001) Ataxin-7 expression analysis in controls and spinocerebellar ataxia type 7 patients. Neurogenetics 3, 83-90
22 Carlson, K.M., Andresen, J.M. and Orr, H.T. (2009) Emerging pathogenic pathways in the spinocerebellar ataxias. Current Opinion in Genetics and Development 19, 247-253
23 Duenas, A.M., Goold, R. and Giunti, P. (2006) Molecular pathogenesis of spinocerebellar ataxias. Brain 129, 1357-1370
24 Shao, J. and Diamond, M.I. (2007) Polyglutamine diseases: emerging concepts in pathogenesis and therapy. Human Molecular Genetics 16, R115-R123
25 Palhan, V.B. et al. (2005) Polyglutamine-expanded ataxin-7 inhibits STAGA histone acetyltransferase activity to produce retinal degeneration. Proceedings of the National Academy of Sciences of the United States of America 102, 8472-8477
26 Zoghbi, H.Y. and Orr, H.T. (2009) Pathogenic mechanisms of a polyglutamine-mediated neurodegenerative disease, spinocerebellar ataxia type 1. Journal of Biological Chemistry 284, 7425-7429
27 Kim, M.O. et al. (2008) Altered histone monoubiquitylation mediated by mutant huntingtin induces transcriptional dysregulation. Journal of Neuroscience 28, 3947-3957
28 Yvert, G. et al. (2001) SCA7 mouse models show selective stabilization of mutant ataxin-7 and similar cellular responses in different neuronal cell types. Human Molecular Genetics 10, 1679-1692
29 Young, J.E. et al. (2009) Polyglutamine-expanded androgen receptor truncation fragments activate a Bax-dependent apoptotic cascade mediated by DP5/Hrk. Journal of Neuroscience 29, 1987-1997
30 Colomer Gould, V.F. et al. (2007) A mutant ataxin-3 fragment results from processing at a site N-terminal to amino acid 190 in brain of Machado-Joseph disease-like transgenic mice. Neurobiology of Disease 27, 362-369
31 Jung, J. et al. (2009) Preventing ataxin-3 protein cleavage mitigates degeneration in a drosophila model of SCA3. Human Molecular Genetics 18, 4843-4852
32 Landles, C. et al. (2010) Proteolysis of mutant huntingtin produces an exon 1 fragment that accumulates as an aggregated protein in neuronal nuclei in Huntington disease. Journal of Biological Chemistry 285, 8808-8823
33 Clemens, L.E. et al. (2010) A14 fast axonal transport of mitochondria is altered in Huntington's disease. Journal of Neurology, Neurosurgery and Psychiatry 81, A5
34 Bennett, E.J. et al. (2007) Global changes to the ubiquitin system in Huntington's disease. Nature 448, 704-708
35 Bence, N.F., Sampat, R.M. and Kopito, R.R. (2001) Impairment of the ubiquitin-proteasome system by protein aggregation. Science 292, 1552-1555
36 Jana, N.R. (2010) Role of the ubiquitin–proteasome system and autophagy in polyglutamine neurodegenerative diseases. Future Neurology 5, 105-112
37 Drouet, V. et al. (2009) Sustained effects of nonallele-specific Huntingtin silencing. Annals of Neurology 65, 276-285
38 Boudreau, R.L. and Davidson, B.L. (2010) RNAi therapeutics for CNS disorders. Brain Research 1338, 112-121
39 Hasholt, L. et al. (2003) Antisense downregulation of mutant huntingtin in a cell model. Journal of Gene Medicine 5, 528-538
40 Bauer, P.O. and Nukina, N. (2009) The pathogenic mechanisms of polyglutamine diseases and current therapeutic strategies. Journal of Neurochemistry 110, 1737-1765
41 Xia, H. et al. (2002) siRNA-mediated gene silencing in vitro and in vivo. Nature Biotechnology 20, 1006-1010

Accession information: doi:10.1017/erm.2011.1; Vol. 14; e3; January 2012
© Cambridge University Press 2012

https://doi.org/10.1017/erm.2011.1 Published online by Cambridge University Press

42 Yamamoto, A., Lucas, J.J. and Hen, R. (2000) Reversal of neuropathology and motor dysfunction in a conditional model of Huntington's disease. Cell 101, 57-66
43 Zu, T. et al. (2004) Recovery from polyglutamine-induced neurodegeneration in conditional SCA1 transgenic mice. Journal of Neuroscience 24, 8853-8861
44 Rodriguez-Lebron, E. and Paulson, H.L. (2005) Allele-specific RNA interference for neurological disease. Gene Therapy 13, 576-581
45 Scholefield, J. and Wood, M.J.A. (2010) Therapeutic gene silencing strategies for polyglutamine disorders. Trends in Genetics 26, 29-38
46 Kubodera, T. et al. (2010) In vivo application of an RNAi strategy for the selective suppression of a mutant allele. Human Gene Therapy 22, 27-34
47 Kubodera, T. et al. (2005) New RNAi strategy for selective suppression of a mutant allele in polyglutamine disease. Oligonucleotides 15, 298-302
48 Nasir, J. et al. (1995) Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. Cell 81, 811-823
49 Dragatsis, I., Levine, M.S. and Zeitlin, S. (2000) Inactivation of hdh in the brain and testis results in progressive neurodegeneration and sterility in mice. Nature Genetics 26, 300-306
50 Van Raamsdonk, J.M. et al. (2005) Loss of wild-type huntingtin influences motor dysfunction and survival in the YAC128 mouse model of Huntington disease. Human Molecular Genetics 14, 1379-1392
51 Persichetti, F. et al. (1996) Differential expression of normal and mutant Huntington's disease gene alleles. Neurobiology of Disease 3, 183-190
52 Ambrose, C.M. et al. (1994) Structure and expression of the Huntington's disease gene: evidence against simple inactivation due to an expanded CAG repeat. Somatic Cell and Molecular Genetics 20, 27-38
53 Matilla, A. et al. (1998) Mice lacking ataxin-1 display learning deficits and decreased hippocampal paired-pulse facilitation. Journal of Neuroscience 18, 5508-5516
54 Jun, K. et al. (1999) Ablation of P/Q-type Ca2 channel currents, altered synaptic transmission, and progressive ataxia in mice lacking the α1A-subunit. Proceedings of the National Academy of Sciences of the United States of America 96, 15245-15250
55 Crespo-Barreto, J. et al. (2010) Partial loss of ataxin-1 function contributes to transcriptional dysregulation in spinocerebellar ataxia type 1 pathogenesis. PLoS Genetics 6, 575-621
56 Lim, J. et al. (2008) Opposing effects of polyglutamine expansion on native protein complexes contribute to SCA1. Nature 452, 713-718
57 Warrick, J.M. et al. (2005) Ataxin-3 suppresses polyglutamine neurodegeneration in drosophila by a ubiquitin-associated mechanism. Molecular Cell 18, 37-48
58 Schmitt, I. et al. (2007) Inactivation of the mouse Atxn3 (ataxin-3) gene increases protein ubiquitination. Biochemical and Biophysical Research Communications 362, 734-739
59 Figueroa, K.P. and Pulst, S.M. (2003) Identification and expression of the gene for human ataxin-2-related protein on chromosome 16. Experimental Neurology 184, 669-678
60 Shen, Y. et al. (2007) Functional architecture of atrophins. Journal of Biological Chemistry 282, 5037-5044
61 Rooms, L. et al. (2006) TBP as a candidate gene for mental retardation in patients with subtelomeric 6q deletions. European Journal of Human Genetics 14, 1090-1096
62 Yeh, S. et al. (2002) Generation and characterization of androgen receptor knockout (ARKO) mice: an in vivo model for the study of androgen functions in selective tissues. Proceedings of the National Academy of Sciences of the United States of America 99, 13498-13503
63 Wang, R.S. et al. (2009) Androgen receptor roles in spermatogenesis and fertility: lessons from testicular cell-specific androgen receptor knockout mice. Endocrine Reviews 30, 119-132
64 Weinberg, M.S. and Wood, M.J.A. (2009) Short non-coding RNA biology and neurodegenerative disorders: novel disease targets and therapeutics. Human Molecular Genetics 18, R27-R39
65 Zamore, P.D. et al. (2000) RNAi:: double-stranded RNA directs the ATP-dependent cleavage of mRNA at 21 to 23 nucleotide intervals. Cell 101, 25-33
66 Castanotto, D. and Rossi, J.J. (2009) The promises and pitfalls of RNA-interference-based therapeutics. Nature 457, 426-433
67 Boudreau, R.L., Monteys, A.M. and Davidson, B.L. (2008) Minimizing variables among hairpin-based RNAi vectors reveals the potency of shRNAs. RNA 14, 1834-1844
68 Grimm, D. et al. (2006) Fatality in mice due to oversaturation of cellular microRNA/short hairpin RNA pathways. Nature 441, 537-541

Accession information: doi:10.1017/erm.2011.1; Vol. 14; e3; January 2012
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https://doi.org/10.1017/erm.2011.1 Published online by Cambridge University Press

69 Boudreau, R.L., Martins, I. and Davidson, B.L. (2008) Artificial microRNAs as siRNA shuttles: improved safety as compared to shRNAs in vitro and in vivo. Molecular Therapy 17, 169-175
70 Denovan-Wright, E.M. et al. (2008) Unexpected off-targeting effects of anti-huntingtin ribozymes and siRNA in vivo. Neurobiology of Disease 29, 446-455
71 Bauer, M. et al. (2008) Prevention of interferon-stimulated gene expression using microRNA-designed hairpins. Gene Therapy 16, 142-147
72 McBride, J.L. et al. (2008) Artificial miRNAs mitigate shRNA-mediated toxicity in the brain: implications for the therapeutic development of RNAi. Proceedings of the National Academy of Sciences of the United States of America 105, 5868-5873
73 Smith, R.A. et al. (2006) Antisense oligonucleotide therapy for neurodegenerative disease. Journal of Clinical Investigation 116, 2290-2296
74 Crooke, S.T. (2004) Progress in antisense technology. Annual Review of Medicine 55, 61-95
75 Gagnon, K.T. et al. (2010) Allele-selective inhibition of mutant huntingtin expression with antisense oligonucleotides targeting the expanded CAG repeat. Biochemistry 49, 10166-10178
76 Boudreau, R.L. et al. (2009) Nonallele-specific silencing of mutant and wild-type huntingtin demonstrates therapeutic efficacy in Huntington's disease mice. Molecular Therapy 17, 1053-1063
77 Harper, S.Q. et al. (2005) RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. Proceedings of the National Academy of Sciences of the United States of America 102, 5820-5825
78 Caplen, N.J. et al. (2002) Rescue of polyglutamine-mediated cytotoxicity by double-stranded RNA-mediated RNA interference. Human Molecular Genetics 11, 175-184
79 Xia, H. et al. (2004) RNAi suppresses polyglutamine-induced neurodegeneration in a model of spinocerebellar ataxia. Nature Medicine 10, 816-820
80 Boado, R.J. et al. (2000) Antisense-mediated down-regulation of the human huntingtin gene. Journal of Pharmacology and Experimental Therapeutics 295, 239-243
81 Rodriguez-Lebron, E. et al. (2005) Intrastriatal rAAV-mediated delivery of anti-huntingtin shRNAs induces partial reversal of disease progression in R6/1 Huntington's disease transgenic mice. Molecular Therapy 12, 618-633
82 Franich, N.R. et al. (2008) AAV vector–mediated RNAi of mutant huntingtin expression is neuroprotective in a novel genetic rat model of Huntington's disease. Molecular Therapy 16, 947-956
83 Machida, Y. et al. (2006) rAAV-mediated shRNA ameliorated neuropathology in Huntington disease model mouse. Biochemical and Biophysical Research Communications 343, 190-197
84 Alves, S. et al. (2010) Silencing ataxin-3 mitigates degeneration in a rat model of Machado–Joseph disease: no role for wild-type ataxin-3? Human Molecular Genetics 19, 2380-2394
85 Abdelgany, A., Wood, M. and Beeson, D. (2003) Allele-specific silencing of a pathogenic mutant acetylcholine receptor subunit by RNA interference. Human Molecular Genetics 12, 2637-2644
86 Gonzalez-Alegre, P. et al. (2003) Toward therapy for DYT1 dystonia: allele-specific silencing of mutant TorsinA. Annals of Neurology 53, 781-787
87 Xia, X. et al. (2006) Allele-specific RNAi selectively silences mutant SOD1 and achieves significant therapeutic benefit in vivo. Neurobiology of Disease 23, 578-586
88 Schwarz, D.S. et al. (2006) Designing siRNA that distinguish between genes that differ by a single nucleotide. PLoS Genetics 2, e140
89 Bilsen, P.H.J. et al. (2008) Identification and allele-specific silencing of the mutant huntingtin allele in Huntington's disease patient-derived fibroblasts. Human Gene Therapy 19, 710-718
90 Pfister, E.L. et al. (2009) Five siRNAs targeting three SNPs may provide therapy for three-quarters of Huntington's disease patients. Current Biology 19, 774-778
91 Ohnishi, Y. et al. (2008) Enhancement of allele discrimination by introduction of nucleotide mismatches into siRNA in allele-specific gene silencing by RNAi. PloS One 3, e2248
92 Lombardi, M.S. et al. (2009) A majority of Huntington's disease patients may be treatable by individualized allele-specific RNA interference. Experimental Neurology 217, 312-319
93 Zhang, Y., Engelman, J. and Friedlander, R.M. (2009) Allele-specific silencing of mutant Huntington's disease gene. Journal of Neurochemistry 108, 82-90
94 Li, Y. et al. (2004) Sequence-dependent and independent inhibition specific for mutant ataxin-3 by small interfering RNA. Annals of Neurology 56, 124-129

Accession information: doi:10.1017/erm.2011.1; Vol. 14; e3; January 2012
© Cambridge University Press 2012

https://doi.org/10.1017/erm.2011.1 Published online by Cambridge University Press

95 Gaspar, C. et al. (1996) Linkage disequilibrium analysis in Machado-Joseph disease patients of different ethnic origins. Human Genetics 98, 620-624
96 Miller, V.M. et al. (2003) Allele-specific silencing of dominant disease genes. Proceedings of the National Academy of Sciences of the United States of America 100, 7195-7200
97 Alves, S. et al. (2008) Allele-specific RNA silencing of mutant ataxin-3 mediates neuroprotection in a rat model of Machado-Joseph disease. PLoS One 3, e3341
98 Scholefield, J. et al. (2009) Design of RNAi hairpins for mutation-specific silencing of ataxin-7 and correction of a SCA7 phenotype. PLoS One 4, e7232
99 Cattaneo, E. et al. (2001) Loss of normal huntingtin function: new developments in Huntington's disease research. Trends in Neurosciences 24, 182-188
100 Hu, J., Matsui, M. and Corey, D.R. (2009) Allele-selective inhibition of mutant huntingtin by peptide nucleic acid-peptide conjugates, locked nucleic acid, and small interfering RNA. Annals of the New York Academy of Sciences 1175, 24-31
101 Kastelein, J.J.P. et al. (2006) Potent reduction of apolipoprotein B and low-density lipoprotein cholesterol by short-term administration of an antisense inhibitor of apolipoprotein B. Circulation 114, 1729-1735
102 Sah, D.W.Y. and Aronin, N. (2011) Oligonucleotide therapeutic approaches for Huntington disease. Journal of Clinical Investigation 121, 500-507
103 Hu, J., Liu, J. and Corey, D.R. (2010) Allele-selective inhibition of huntingtin expression by switching to an miRNA-like RNAi mechanism. Chemistry and Biology 17, 1183-1188
104 Kim, D.H. and Rossi, J.J. (2003) Coupling of RNAi-mediated target downregulation with gene replacement. Antisense and Nucleic Acid Drug Development 13, 151-155
105 Kim, D.H. et al. (2008) MicroRNA-directed transcriptional gene silencing in mammalian cells. Proceedings of the National Academy of Sciences of the United States of America 105, 16230-16235
106 Younger, S.T. and Corey, D.R. (2011) Transcriptional gene silencing in mammalian cells by miRNA mimics that target gene promoters. Nucleic Acids Research 39, 5682-5691
107 Hawkins, P.G. et al. (2009) Promoter targeted small RNAs induce long-term transcriptional gene silencing in human cells. Nucleic Acids Research 37, 2984-2995
108 Morris, K.V. et al. (2008) Bidirectional transcription directs both transcriptional gene activation and suppression in human cells. PLoS Genetics 4, e1000258
109 Han, J., Kim, D. and Morris, K.V. (2007) Promoter-associated RNA is required for RNA-directed transcriptional gene silencing in human cells. Proceedings of the National Academy of Sciences of the United States of America 104, 12422-12427
110 Li, L.B. et al. (2008) RNA toxicity is a component of ataxin-3 degeneration in drosophila. Nature 453, 1107-1111
111 de Mezer, M. et al. (2011) Mutant CAG repeats of huntingtin transcript fold into hairpins, form nuclear foci and are targets for RNA interference. Nucleic Acids Research 39, 3852-3863
112 Wheeler, T.M. et al. (2009) Reversal of RNA dominance by displacement of protein sequestered on triplet repeat RNA. Science 325, 336-339
113 Todd, P.K. and Paulson, H.L. (2010) RNA-mediated neurodegeneration in repeat expansion disorders. Annals of Neurology 67, 291-300
114 Dickson, A.M. and Wilusz, C.J. (2010) Repeat expansion diseases: when a good RNA turns bad. Wiley Interdisciplinary Reviews – RNA 1, 173-192
115 Roshan, R. et al. (2009) MicroRNAs: novel therapeutic targets in neurodegenerative diseases. Drug Discovery Today 14, 1123-1129
116 Johnson, R. et al. (2007) A microRNA-based gene dysregulation pathway in Huntington's disease. Neurobiology of Disease 29, 438-445
117 Lee, Y. et al. (2008) miR-19, miR-101 and miR-130 co-regulate ATXN1 levels to potentially modulate SCA1 pathogenesis. Nature Neuroscience 11, 1137-1139
118 Lundstrom, K. (2011) Micro-RNA in disease and gene therapy. Current Drug Discovery Technologies 8, 76-86
119 Bilen, J., Liu, N. and Bonini, N.M. (2006) A new role for microRNA pathways: modulation of degeneration induced by pathogenic human disease proteins. Cell Cycle 5, 2835-2838
120 Schaefer, A. et al. (2007) Cerebellar neurodegeneration in the absence of microRNAs. Journal of Experimental Medicine 204, 1553-1558
121 Giraldez, A.J. et al. (2005) MicroRNAs regulate brain morphogenesis in zebrafish. Science 308, 833-838
122 Lesnik, J.K. and Antes, T.J. (2010) Permanent knockdown of microRNAs using lentivectors. BioTechniques 48, 321-323

Accession information: doi:10.1017/erm.2011.1; Vol. 14; e3; January 2012
© Cambridge University Press 2012

https://doi.org/10.1017/erm.2011.1 Published online by Cambridge University Press

123 Lennox, K. and Behlke, M. (2011) Chemical modification and design of anti-miRNA oligonucleotides. Gene Therapy 18, 1111-1120
124 Elmén, J. et al. (2008) LNA-mediated microRNA silencing in non-human primates. Nature 452, 896-899
125 Obad, S. et al. (2011) Silencing of microRNA families by seed-targeting tiny LNAs. Nature Genetics 43, 371-378
126 Worm, J. et al. (2009) Silencing of microRNA-155 in mice during acute inflammatory response leads to derepression of c/ebp beta and down-regulation of G-CSF. Nucleic Acids Research 37, 5784-5792
127 Jadhav, V.M., Scaria, V. and Maiti, S. (2009) Antagomirzymes: oligonucleotide enzymes that specifically silence microRNA function. Angewandte Chemie International Edition 48, 2557-2560
128 Broderick, J.A. and Zamore, P.D. (2011) MicroRNA therapeutics. Gene Therapy advance online publication, 28 April 2011; doi:10.1038/gt.2011.50
129 Cao, W. et al. (2005) DNA constructs designed to produce short hairpin, interfering RNAs in transgenic mice sometimes show early lethality and an interferon response. Journal of Applied Genetics 46, 217-225
130 Sledz, C.A. et al. (2003) Activation of the interferon system by short-interfering RNAs. Nature Cell Biology 5, 834-839
131 Jackson, A.L. and Linsley, P.S. (2010) Recognizing and avoiding siRNA off-target effects for target identification and therapeutic application. Nature Reviews Drug Discovery 9, 57-67
132 Jackson, A.L. et al. (2003) Expression profiling reveals off-target gene regulation by RNAi. Nature Biotechnology 21, 635-637
133 Jackson, A.L. et al. (2006) Widespread siRNA "off-target" transcript silencing mediated by seed region sequence complementarity. RNA 12, 1179-1187
134 Persengiev, S.P., Zhu, X. and Green, M.R. (2004) Nonspecific, concentration-dependent stimulation and repression of mammalian gene expression by small interfering RNAs (siRNAs). RNA 10, 12-18
135 Naito, Y. et al. (2004) siDirect: highly effective, target-specific siRNA design software for mammalian RNA interference. Nucleic Acids Research 32 (Suppl. 2), W124-W129
136 Kittler, R. et al. (2007) Genome-wide resources of endoribonuclease-prepared short interfering RNAs for specific loss-of-function studies. Nature Methods 4, 337-344
137 Bramsen, J.B. et al. (2010) A screen of chemical modifications identifies position-specific modification by UNA to most potently reduce siRNA off-target effects. Nucleic Acids Research 38, 5761-5773
138 Fedorov, Y. et al. (2006) Off-target effects by siRNA can induce toxic phenotype. RNA 12, 1188-1196
139 Jackson, A.L. et al. (2006) Position-specific chemical modification of siRNAs reduces "off-target" transcript silencing. RNA 12, 1197-1205
140 Hornung, V. et al. (2005) Sequence-specific potent induction of IFN-α by short interfering RNA in plasmacytoid dendritic cells through TLR7. Nature Medicine 11, 263-270
141 Judge, A.D. et al. (2006) Design of noninflammatory synthetic siRNA mediating potent gene silencing in vivo. Molecular Therapy 13, 494-505
142 Sioud, M. (2005) Induction of inflammatory cytokines and interferon responses by double-stranded and single-stranded siRNAs is sequence-dependent and requires endosomal localization. Journal of Molecular Biology 348, 1079-1090
143 Kleinman, M.E. et al. (2008) Sequence-and target-independent angiogenesis suppression by siRNA via TLR3. Nature 452, 591-597
144 Khan, A.A. et al. (2009) Transfection of small RNAs globally perturbs gene regulation by endogenous microRNAs. Nature Biotechnology 27, 549-555
145 Dessy, A. and Gorman, J.M. (2011) The emerging therapeutic role of RNA interference in disorders of the central nervous system. Clinical Pharmacology and Therapeutics 89, 450-454
146 Eberling, J.L. et al. (2008) Results from a phase I safety trial of hAADC gene therapy for Parkinson disease. Neurology 70, 1980-1983
147 Naldini, L. et al. (1996) Efficient transfer, integration, and sustained long-term expression of the transgene in adult rat brains injected with a lentiviral vector. Proceedings of the National Academy of Sciences of the United States of America 93, 11382-11388
148 Dorn, G. et al. (2004) siRNA relieves chronic neuropathic pain. Nucleic Acids Research 32, e49
149 Alvarez-Erviti, L. et al. (2011) Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. Nature Biotechnology 29, 341-345
150 Agrawal, A. et al. (2009) Functional delivery of siRNA in mice using dendriworms. ACS Nano 3, 2495-2504

Accession information: doi:10.1017/erm.2011.1; Vol. 14; e3; January 2012
© Cambridge University Press 2012

https://doi.org/10.1017/erm.2011.1 Published online by Cambridge University Press

151 Luo, M.C. et al. (2005) An efficient intrathecal delivery of small interfering RNA to the spinal cord and peripheral neurons. Molecular Pain 1, 29-36
152 Vaishnaw, A.K. et al. (2010) A status report on RNAi therapeutics. Silence 1, 1-13
153 Cattaneo, E., Zuccato, C. and Tartari, M. (2005) Normal huntingtin function: an alternative approach to Huntington's disease. Nature Reviews Neuroscience 6, 919-930
154 Wang, L. et al. (2006) Histone deacetylase-associating atrophin proteins are nuclear receptor corepressors. Genes and Development 20, 525-530
155 Goold, R. et al. (2007) Down-regulation of the dopamine receptor D2 in mice lacking ataxin 1. Human Molecular Genetics 16, 2122-2134
156 Ralser, M. et al. (2005) An integrative approach to gain insights into the cellular function of human ataxin-2. Journal of Molecular Biology 346, 203-214
157 Kiehl, T.R. et al. (2006) Generation and characterization of Sca2 (ataxin-2) knockout mice. Biochemical and Biophysical Research Communications 339, 17-24
158 Huynh, D.P. et al. (2009) Dissociated fear and spatial learning in mice with deficiency of ataxin-2. PloS One 4, e6235
159 Evert, B.O. et al. (1999) High level expression of expanded full-length ataxin-3 in vitro causes cell death and formation of intranuclear inclusions in neuronal cells. Human Molecular Genetics 8, 1169-1176
160 Helmlinger, D. et al. (2004) Ataxin-7 is a subunit of GCN5 histone acetyltransferase-containing complexes. Human Molecular Genetics 13, 1257-1265
161 Shah, A.G. et al. (2009) Transcriptional dysregulation of TrkA associates with neurodegeneration in spinocerebellar ataxia type 17. Human Molecular Genetics 18, 4141-4152
162 van Roon-Mom, W.M.C. et al. (2005) TATA-binding protein in neurodegenerative disease. Neuroscience 133, 863-872
163 Zimmermann, T.S. et al. (2006) RNAi-mediated gene silencing in non-human primates. Nature 441, 111-114
164 Henry, S. et al. (2000) Chemically modified oligonucleotides exhibit decreased immune stimulation in mice. Journal of Pharmacology and Experimental Therapeutics 292, 468-479
165 Kunze, D., Kraemer, K. and Fuessel, S. (2010) Antisense oligonucleotides: insights from preclinical studies and clinical trials. In RNA Technologies and their Applications (Erdmann, V.A. and Barciszewski, J., eds), pp. 285-303, Springer, Berlin Heidelberg

Further reading, resources and contacts

Di Prospero, N.A. and Fischbeck, K.H. (2005) Therapeutics development for triplet repeat expansion diseases. Nature Reviews Genetics 6, 756-767

An overview of therapeutic strategies for triplet repeat disorders, focusing primarily on non-RNA-mediated approaches to target cellular pathology.

Kim, D.H. and Rossi, J.J. (2007) Strategies for silencing human disease using RNA interference. Nature Reviews Genetics 8, 173-184

A comprehensive review of delivery strategies for RNA-based gene therapy.

La Spada, A.R. and Taylor, J.P. (2010) Repeat expansion disease: progress and puzzles in disease pathogenesis. Nature Reviews Genetics 11, 247-258

An overview of the current understanding of disease mechanisms in repeat expansion diseases.

Malecová, B. and Morris, K.V. (2010) Transcriptional gene silencing mediated by non-coding RNAs. Current Opinion in Molecular Therapeutics 12, 214-222

This review offers a more thorough understanding of the endogenous and exogenous mechanisms underlying mammalian transcriptional gene silencing.

Takahashi, M. et al. (2010) Tailor-made RNAi knockdown against triplet repeat disease-causing alleles. Proceedings of the National Academy of Sciences of the United States of America 107, 21731-21736

An interesting study describing a pulldown method to rapidly identify SNPs linked to expanded alleles, in order to facilitate the efficient development of allele-specific RNAi-based therapy.

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Features associated with this article

Figures

Figure 1. Mechanisms of polyQ toxicity.
Figure 2. Principles of allele-specific, non-allele-specific, and gene knockdown and replacement therapies.
Figure 3. RNA interference-based therapies make use of the endogenous mammalian gene silencing pathway.

Tables

Table 1. PolyQ gene function, phenotypic effect of loss of function, and implications for choice of gene silencing therapy.
Table 2. Comparison of RNAi- and AON-based gene silencing effectors.

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