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Brief Communication

Allele-Selective Inhibition of Huntingtin Expression by Switching to an miRNA-like RNAi Mechanism

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SUMMARY

Inhibiting expression of huntingtin (HTT) protein is a promising strategy for treating Huntington's disease (HD), but indiscriminant inhibition of both wild-type and mutant alleles may lead to toxicity. An ideal silencing agent would block expression of mutant HTT while leaving expression of wild-type HTT intact. We observe that fully complementary duplex RNAs targeting the expanded CAG repeat within HTT mRNA block expression of both alleles. Switching the RNAi mechanism toward that used by miRNAs by introducing one or more mismatched bases into these duplex RNAs leads to potent (<10 nM) and highly selective (>30-fold relative to wild-type HTT) inhibition of mutant HTT expression in patient-derived cells. Potent, allele selective inhibition of HTT by mismatched RNAs provides a new option for developing HD therapeutics.

INTRODUCTION

Huntington's disease (HD) is a neurodegenerative disorder with an incidence of 5–10 per 100,000 individuals in Europe and North America (Walker, 2007). HD is characterized by progressive neurodegeneration resulting in chorea, dystonia, and cognitive or psychiatric disturbances. Symptoms worsen until death, 10–20 years after disease onset. Genetic tests and palliative treatments exist, but there are no curative therapies. Effective therapeutic strategies that delay the onset of HD remain a major unmet need.

HD is caused by a dominant heterozygous expansion of a CAG trinucleotide repeat that encodes multiple glutamine residues within the gene encoding huntingtin (HTT) protein (MacDonald et al., 1993). The mutant HTT allele in patients with HD contains tracts of more than 39 consecutive glutamine codons. Most patients have 50 repeats or less, but some patients can have as many as 120. Higher numbers of repeats correlate with earlier onset of disease (Kremer et al., 1994; Duyao et al., 1993). Although the exact molecular defects caused by mutant HTT expression are not definitively known, aggregation of polyglutamine fragments of HTT induces plaque formation (Sanchez et al., 2003). HTT is expressed in many tissues, but the central nervous system and brain are especially sensitive to expression of mutant HTT, with striatal neurons suffering the most degeneration (Reddy et al., 1998).

Developing agents to lower levels of mutant HTT in patients offers one strategy for developing medications to treat HD (Pfister and Zamore, 2009). Lower HTT levels should delay or prevent aggregation of polyglutamine fragments, slow plaque formation, and reduce damage to cells. This hypothesis has been tested in several animal models using both antisense oligonucleotides and duplex RNAs, and reducing levels of mutant HTT has been shown to alleviate symptoms and prolong survival (Harper et al., 2005; DiFiglia et al., 2007; Boudreau et al., 2009; Gagnon, 2010). These data suggest that controlling mutant HTT in patients would be a major therapeutic advance.

Typically, agents that silence gene expression block expression of both alleles of the target gene. However, wild-type HTT may play a role in normal adult function (Nasir et al., 1995; White et al., 1997), and strategies that reduce expression of both the wild-type and mutant alleles may cause side effects in patients. Although it is possible that an agent that inhibits both wild-type and mutant HTT might be both safe and effective (Drouet et al., 2009; Boudreau et al., 2009), an ideal agent would selectively reduce mutant HTT expression but not alter wild-type protein levels. Because of the lack of curative treatments for HD, it is essential that both allele-selective and nonallele-selective approaches for reducing mutant HTT levels be pursued.

Strategies have been developed that achieve allele-selective inhibition of HTT expression. One approach takes advantage of the fact that the mutant HTT allele often contains single-nucleotide polymorphisms that distinguish it from the wild-type allele, allowing short-interfering RNAs (siRNAs) to selectively target the mutant gene (Miller et al., 2003; Schwarz et al., 2006; van Bilsen et al., 2008; Pfister et al., 2009; Zhang et al., 2009). The identity of SNPs varies, with 86% of European Caucasian patients sharing 26 different SNPs (Lombardi et al., 2009), but some SNPs are common, and only five duplex RNAs would be sufficient to treat 75% of patients with HD (Schwarz et al., 2006). Although targeting SNPs is promising, the need to develop several different agents complicates development and suggests a continuing need for new strategies for silencing HD.

Another strategy involves single-stranded oligomers complementary to the expanded CAG repeat within HTT mRNA that achieve potent and selective inhibition of HTT expression (Hu et al., 2009a, 2009b, 2009c). This strategy would require just

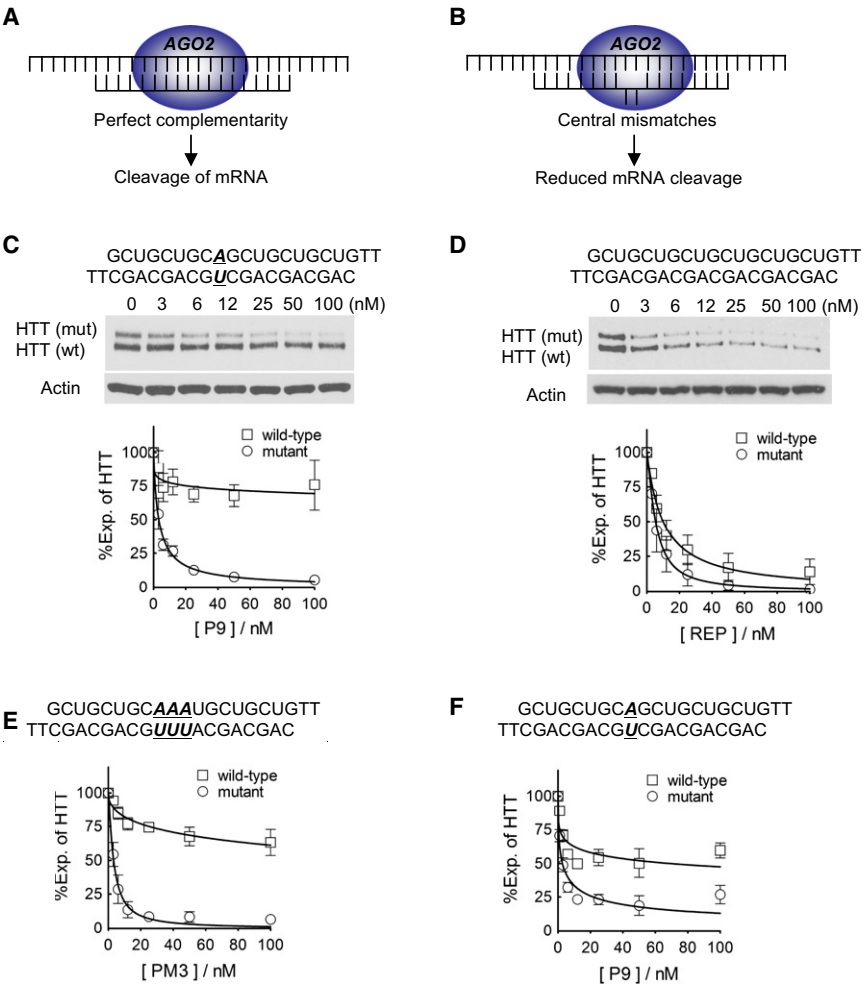

Figure 1. Allele-Selective Inhibition by Mismatch-Containing Duplex RNAs

Schemes showing recognition of a (A) perfectly complementary duplex leading to cleavage of mRNA and (B) mismatch-containing duplex leading to inhibition of translation. Effect on HTT expression from adding duplex RNAs (C) P9, (D) REP, and (E) PM3 to GM04281 cells, or (F) P9 to GM04717 cells. Data describing the selectivities and potencies of other duplexes can be found in Table 1.

See also Figure S1. Error bars for data derived from western analysis are standard error of the mean (SEM).

one oligomer to treat the entire population of patients with HD. Oligomers that target the long (250 repeat) CUG repeat within the 3′ untranslated region (3′-UTR) of DM protein kinase, the cause of myotonic dystrophy, have also been shown to be biologically active (Wheeler et al., 2009). However, after testing several different chemically modified oligomers complementary to the CAG repeat in HTT mRNA, it has been difficult to improve upon the potencies and selectivities that were observed initially (Gagnon et al., 2010).

Developing highly potent and selective methods for silencing the mutant allele of HTT remains challenging. Based on the potency of duplex RNA and the length of CAG repeat, we asked whether we could achieve the selectivity by targeting CAG repeat region using siRNA. We observed that these siRNAs were potent inhibitors of HTT but showed little selectively between the mutant and wild-type alleles (Hu et al., 2009a, 2009c).

RNAi can function through two distinct mechanisms (Filipowicz et al., 2008; Kurreck 2009). If the duplex RNA is fully complementary to its mRNA target, human argonaute 2 (AGO2) protein cleaves the mRNA at the phosphodiester linkage opposite siRNA bases 10 and 11 (Figure 1A) (Liu et al., 2004). This mechanism is used by the siRNAs commonly used in laboratories for gene silencing.

However, mismatches between the duplex RNA and mRNA target disrupt interactions at the catalytic center of AGO2 and make cleavage less efficient (Figure 1B) (Du et al., 2005; Wang et al., 2008). Gene silencing can still occur but may involve inhibition of ribosome-mediated translation or reduced mRNA stability rather than cleavage of the mRNA target. This mechanism is commonly used by naturally occurring miRNAs to inhibit expression of endogenous genes and regulate cell physiology. In addition the expanded CAG repeat offers multiple target sequences for finding complementary duplex RNAs, and it is known that the binding of multiple mismatch-containing duplex RNAs to mRNA can yield synergistic increases in potency for miRNAs (Grimson et al., 2007). The miRNA-like mechanism is similar to the mechanism of single-stranded PNAs, which also block translation rather than cause mRNA cleavage (Knudsen and Nielsen, 1996), raising the possibility that miRNA-like duplexes might be better candidates for achieving allele selectivity. We hypothesized that deliberately switching the RNAi mechanism would be advantageous for discriminating among closely related RNA targets and achieving allele-selective regulation of disease genes.

Here, we test mismatch-containing duplex RNAs complementary to the CAG repeat. We find that they are potent, versatile, and selective agents for blocking expression of the mutant HTT allele.

RESULTS AND DISCUSSION

We transfected duplex RNA P9 (single mismatch at base 9 within the guide strand/antisense relative to the mRNA target) (Table 1) into a patient-derived fibroblast cell line GM04281 (69 CAG repeats/mutant; 17 repeats/wild-type allele). P9 possessed high selectively, >31-fold, for inhibition of mutant versus wild-type HTT (Figure 1C). Fully complementary RNA REP and sister compounds REPC and REPU that were shifted to start with C or U showed selectivities of less than 2- to 3-fold (Figure 1D; see

Table 1. siRNAs Targeting the HTT CAG Repeat Region

RNA	Sequence	Position of mismatch	Tm,°C	mut IC_{50} (nM)	wt IC_{50} (nM)	Selectivity
siRNA Fully Complementary to CAG Repeat Region (only guide strand is shown)						
REP	GCUGCUGCUGCUGCUGCUGTT		86.8	5.0 ± 3.0	13 ± 5.0	3
REPC	CUGCUGCUGCUGCUGCUGCTT		86.6	5.4 ± 1.6	12 ± 2.6	2
REPU	UGCUGCUGCUGCUGCUGCUTT		86.5	5.6 ± 1.1	9.8 ± 1.2	2
siRNA Containing One Mismatched Base on Both Strands (only guide strand is shown)						
P4	GCU*A*CUGCUGCUGCUGCUGTT	4	84.0	4.1 ± 2.0	16 ± 7.4	4
P5	GCUG*A*UGCUGCUGCUGCUGTT	5	83.2	14 ± 2.9	>100	>7
P6	GCUGC*A*GCUGCUGCUGCUGTT	6	85.1	No inhibition	No inhibition	-
P7	GCUGCU*A*CUGCUGCUGCUGTT	7	82.7	13 ± 5.6	53 ± 13	4
P8	GCUGCUG*A*UGCUGCUGCUGTT	8	83.8	5.1 ± 0.7	>100	>20
P9	GCUGCUGC*A*GCUGCUGCUGTT	9	86.7	3.2 ± 0.9	>100	>31
P10	GCUGCUGCU*A*CUGCUGCUGTT	10	83.5	4.0 ± 2.8	>100	>25
P10R	GCUGCUGCU*U*CUGCUGCUGTT	10	78.0	3.5 ± 2.2	>100	>27
P11	GCUGCUGCUG*A*UGCUGCUGTT	11	83.7	2.7 ± 0.8	>100	>37
P12	GCUGCUGCUGC*A*GCUGCUGTT	12	85.6	3.7 ± 1.9	33 ± 12	9
P13	GCUGCUGCUGCU*A*CUGCUGTT	13	82.8	5.3 ± 1.1	26 ± 8.4	5
P16	GCUGCUGCUGCUGCU*A*CUGTT	16	76.4	1.0 ± 0.8	7.0 ± 1.3	7
siRNA Containing Multiple Mismatched Bases on Both (only guide strand is shown)						
P910	GCUGCUGC*AA*CUGCUGCUGTT	9,10	83.5	4.5 ± 0.9	>100	>22
PM3	GCUGCUGC*AAA*UGCUGCUGTT	9,10,11	79.9	3.2 ± 1.0	>100	>31
PM4	GCUGCUG*AAAA*UGCUGCUGTT	8,9,10,11	76.4	4.8 ± 1.6	>100	>21
RM3	GCU*A*CUGCU*A*CUGCU*A*CUGTT	4,10,16	83.0	63 ± 42	>100	>2
RM4	GC*A*GCUG*U*UGCU*A*CUG*U*UGTT	3,8,13,17	78.0	No inhibition	No inhibition	-
siRNA Containing One or Two Mismatched Bases on Only One Strand						
P9a	GCUGCUGCUGCUGCUGCUGTT TTCGACGACG*U*CGACGACGAC	9	79.4	2.0 ± 0.6	8.9 ± 1.3	4
P10a	GCUGCUGCUGCUGCUGCUGTT TTCGACGACGA*U*GACGACGAC	10	80.8	5.4 ± 1.4	27 ± 12	5
P910a	GCUGCUGCUGCUGCUGCUGTT TTCGACGACG*UU*GACGACGAC	9,10	77.6	2.7 ± 0.5	9.9 ± 2.0	4
P9b	GCUGCUGC*A*GCUGCUGCUGTT TTCGACGACGACGACGACGAC	9	80.0	2.3 ± 1.5	>100	>43
P10b	GCUGCUGCU*A*CUGCUGCUGTT TTCGACGACGACGACGACGAC	10	77.6	6.8 ± 1.5	>100	>15
P10R –b	GCUGCUGCU*U*CUGCUGCUGTT TTCGACGACGACGACGACGAC	10	75.4	2.3 ± 0.5	>100	>43
P11b	GCUGCUGCUG*A*UGCUGCUGTT TTCGACGACGACGACGACGAC	11	77.5	10 ± 2.4	>100	>10
P910b	GCUGCUGC*AA*CUGCUGCUGTT TTCGACGACGACGACGACGAC	9,10	74.1	6.6 ± 0.6	>100	>15
REPC-b	CUGCUGCUG*A*UGCUGCUGCTT TTGACGACGACGACGACGACG	10	77.2	7.5 ± 2.1	>100	>13
REPU-b	UGCUGCUGC*A*GCUGCUGCUTT TTACGACGACGACGACGACGA	10	80.2	4.0 ± 1.2	>100	>25
Noncomplementary Control siRNA						
CM	GCUAUACCAGCGUCGUCAUTT		80.0	-	-	-
siRNA Tested on Fibroblasts of Patients with HD with Shorter CAG Repeat						
P9	In GM04719, 44 mut/15 wt			4.5 ± 1.3	>100	>22
P910	In GM04719, 44 mut/15 wt			8.9 ± 2.2	>100	>11
P9	In GM04717, 41 mut/20 wt			2.6 ± 0.6	71 ± 43	27
P910	In GM04717, 41 mut/20 wt			4.2 ± 0.8	57 ± 16	13

The guide strand is shown from 5′ to 3′. Mismatched bases are underlined and in italics. Both strands of the duplexes containing two TT overhang. siRNAs were tested in fibroblast GM04281 of patients with HD (mutant [mut] allele/69 CAG, wild type [wt]/17 CAG repeats) unless otherwise noted. Selectivity is calculated by dividing the IC_{50} for inhibition of wild-type HTT versus the IC_{50} for inhibiting expression of the mutant HTT protein.

Figure S1A available online). These data demonstrated that mismatch-containing RNAs widen the window for achieving selective inhibition.

We tested duplexes containing mismatches placed sequentially throughout the RNA duplex (Table 1; Figures S1B and S1C). siRNAs P8, P9, P10, P10R, and P11 containing centrally located mismatches yielded greater selectivity for inhibition of mutant versus wild-type HTT expression (>20- to >37-fold) than duplexes P4, P5, P6, P7, P12, P13, and P16 with mismatches closer to the 5′ or 3′ termini (4- to 9-fold). Mutations

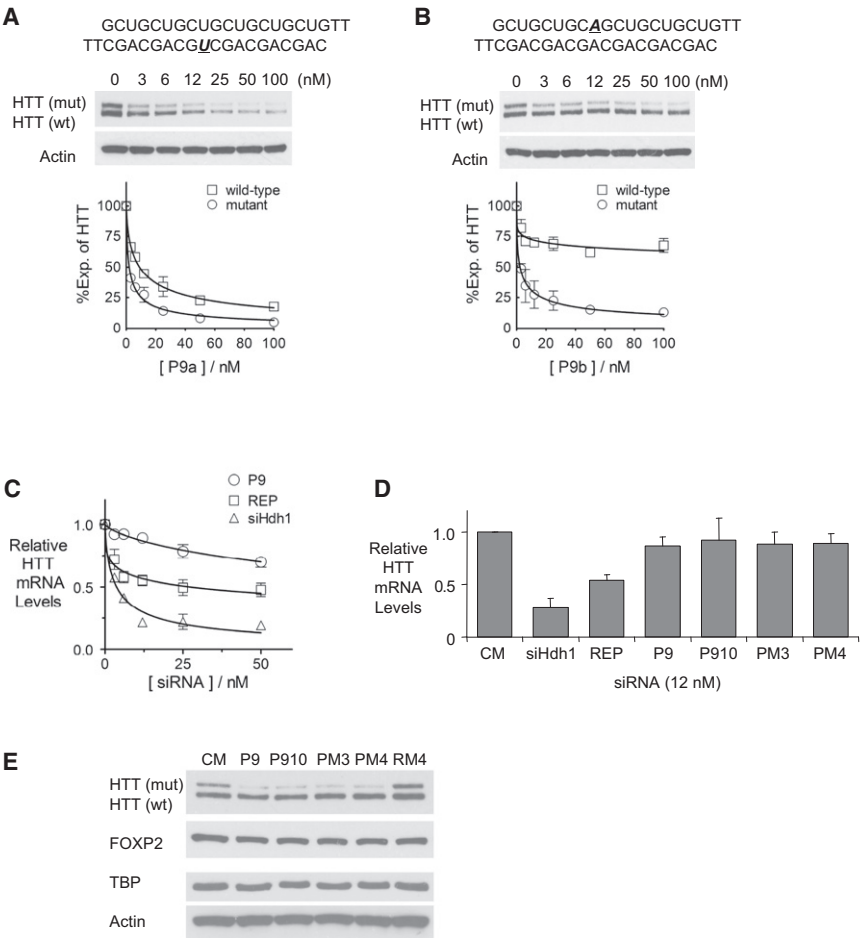

Figure 2. Mechanism of Allele-Selective RNAs

(A and B) Effect on mutant and wild-type HTT expression after treatment with duplex RNAs that contain a mismatched base in either the (A) passenger strand, P9a, or (B) guide strand, P9b.

(C) qPCR analysis of effect on HTT mRNA levels after treatment of increasing amount of siRNAs siHdh1, REP, and P9.

(D) Effect of varied siRNAs (12 nM) on HTT mRNA levels measured by qPCR. (E) Effect of allele-selective duplex RNAs (25 nM) on expression of other genes containing regions with CAG repeats. CM, a duplex RNA not complementary to HTT mRNA; siHdh1, an siRNA that is complementary to HTT mRNA outside of the CAG repeat region (Omi et al., 2005).

See also Figure S2. Error bars for data derived from western analysis are standard error of the mean (SEM). Error bars for qPCR data are standard deviation.

within the RNA duplex disrupt cleavage of the mRNA by RISC (Du et al., 2005; Wang et al., 2008), and our data indicated that shifting the mechanism for RNAi away from mRNA cleavage improves selectivity.

We tested duplexes containing several mismatches to establish the breadth of modifications that might be tolerated. When multiple mismatches are spread throughout the RNA duplex, potencies decrease substantially (Figure S1D). However, when two, three, or four contiguous mismatches are clustered within the central portion of the siRNA, we observed potent and selective inhibition of mutant HTT (Figures 1E; S1E). The ability to introduce as many as four mismatches provides great flexibility for optimizing selective compounds for in vivo investigation and offers additional evidence for a mechanism that does not depend on RISC-mediated cleavage.

Half of patients with HD have mutant alleles with <45 CAG repeats (Duyao et al., 1993; MacDonald et al., 1993). To determine whether our strategy might be applied to a broad range of patients with HD, we tested mismatch-containing duplex RNAs in GM04719 (44 repeats/mutant; 15 repeats/wild-type allele) and GM04717 (41 repeats/mutant; 20 repeats/wild-type allele) patient-derived fibroblast cells. Previously, we had observed that single-stranded PNAs could achieve >1.8- and >1.5-fold selectivities for GM04719 and GM04717 cells, respectively. Using mismatch-containing duplex RNAs, we observed robust selectivities (>10- to 20-fold) and potencies for inhibiting mutant HTT (<10 nM) in both cell lines (Figure 1F and Table 1; Figures 1F and S1G), demonstrating substantial allele-selective inhibition across the full spectrum of CAG repeat lengths encountered in patients.

Duplex RNAs consist of a guide strand (antisense relative to the mRNA) and a passenger strand (sense relative to the mRNA). The guide strand is complementary to the target mRNA, is loaded into the RISC complex, binds to the mRNA target, and should be more sensitive to the introduction of mutations than the passenger strand. Duplexes P9a, P10a, and P910a with mutations on the passenger strand possessed relatively low selectivities (4- or 5-fold) (Figure 2A; Figure S2A). Duplexes P9b, P10b, P10R/b, P11b, and P910b with mutations on the guide strand had selectivities up to >43-fold (Figure 2B; Figures S2B and S2C), indicating that the mechanism of selectivity requires mutation of the guide strand.

We used quantitative PCR (qPCR) to evaluate levels of HTT mRNA after addition of mismatch-containing siRNA P9, fully complementary siRNA REP, and siRNA siHdh1 (Omi et al., 2005) that recognizes HTT mRNA at a sequence outside of the CAG repeat region. Relative to addition of REP or siHdh1, addition of P9 caused a significantly lower reduction in HTT mRNA levels (Figure 2C). Mismatch-containing RNAs P910, PM3, and PM4 also resulted in little alteration of HTT mRNA levels (Figure 2D). The finding that mismatch-containing duplex RNAs yield little decrease of HTT mRNA is consistent with a mechanism of action that involves blocking translation rather than mRNA cleavage.

Several physiologically important genes contain CAG repeats, and inhibiting their expression would be detrimental. We examined expression of two important repeat-containing genes,

TATA box-binding protein (TBP) (19 CAG repeats) and FOXP2 (40 glutamines encoded by a mixed CAG and CAA repeats). We observed that expression of these proteins was not inhibited by a panel of duplex RNAs that were added at 25 nM, a concentration substantially above their IC_{50} values for inhibiting mutant HTT (Figure 2E). We had previously observed that single-stranded oligomers targeting the CAG repeat that reduce levels of mutant HTT protein also did not affect expression of a panel of CAG repeat-containing genes (Hu et al., 2009c).

There are no curative treatments of HD, and strategies for reducing expression of HTT offer a direct approach toward mitigating or preventing the course of the disease. Improved delivery technologies (Smith et al., 2006; De Souza et al., 2009) are making nucleic acids a promising approach for neurodegenerative diseases. A Phase I clinical trial for amyotrophic lateral sclerosis (ALS) using an antisense oligonucleotide delivered directly into the central nervous system has recently been initiated (http://isispharm.com). Given the obvious potential for benefits to patients from reducing HTT levels and improving methods for nucleic acid delivery to the brain, approaches that use duplex RNAs or antisense oligonucleotides to silence HTT expression are a promising approach toward meeting a major unmet therapeutic need.

It is possible that nonallele-specific inhibition of both wild-type and mutant HTT protein may be an adequate therapeutic approach. There may be a window where inhibition of the mutant allele will be sufficient to prevent or reverse formation of harmful aggregation of polyglutamine fragments, whereas equivalent inhibition of the wild-type protein preserves sufficient HTT for essential normal functions. However, agents that preferentially inhibit expression of mutant HTT might widen this therapeutic window and improve treatment outcomes.

In our previous studies we examined inhibition of HTT expression by single-stranded oligomers targeting the CAG repeat (Hu et al., 2009a, 2009b, 2009c). Our data were promising, with significant allele selectivity being achieved by several different chemically modified oligomers. Locked nucleic acid oligomers (LNAs), a chemistry already being tested in the clinical Phase II trials for treatment of other diseases (Koch et al., 2008), showed up to 6-fold allele selectivity and provide a promising starting point for further development (Hu et al., 2009c). However, after exhaustively testing peptide nucleic acid (PNA), LNA, (S)-cET bridged nucleic acid, carba-LNA, ethylene nucleic acid (ENA), altritol nucleic acid (ANA), 2′-O-methoxyethyl (MOE), 2′-fluoro, and 2′-fluoroarabino nucleic acid (FANA) oligomers, we were unable to identify any compound with better than 8-fold allele-selectivity (Gagnon et al., 2010).

Our most effective single-stranded oligonucleotides inhibited expression by blocking gene translation rather than causing degradation of HTT mRNA. Because mismatched RNAs can also function through the RNAi pathway by blocking translation, we tested them and observed high levels of allele selectivity. Importantly, this selectivity was not a property of only one or two compounds. Several different arrangements of mismatched bases were potent and selective inhibitors of gene expression, including one compound with four contiguous mismatched nucleotides. These RNA duplexes demonstrate an ample supply of lead compounds to optimize for the potency, allele selectivity, low toxicity, and biodistribution necessary for successful in vivo application.

SIGNIFICANCE

Developing curative treatments for HD remains an urgent unmet medical need. Reducing the amount of mutant HTT would directly target the agent causing HD, and duplex RNAs and antisense oligonucleotides have proven to be effective in engineered animal models. The challenge now is to improve the properties of anti-HTT nucleic acids sufficiently to enable clinical trials in humans. One important goal for improvement is identification of compounds that preferentially reduce levels of mutant HTT while preserving physiologically essential levels of wild-type HTT.

Mismatch-containing RNAs reduce expression of mutant HTT robustly, with low nanomolar potencies and selectivities greater than 30 fold. Selective inhibition can be achieved in cell lines with mutant alleles containing low numbers of repeats, extending the approach to cell culture models representing almost all of the human patient population. The fact that several different compounds are potent and selective offers several starting points for the medicinal chemistry necessary to create molecules that will have optimal potency, selectivity, and biodistribution.

Beyond potential therapeutic application to HD, our work has implications for understanding recognition of RNA sequences during RNAi. The basic target sequence—multiple CAGs—is the same regardless of whether the repeat is expanded. The RISC complexes formed by duplex RNAs must sense structural variations or relatively modest differences in repeat length. This sensitivity can be translated into substantial improvements in recognition by a diverse group of mismatch-containing RNA duplexes. Introducing mismatches may allow gene silencing to be fine-tuned to achieve optimal results at specific RNA target sequences and structures.

EXPERIMENTAL PROCEDURES

Full details of materials and experimental procedures used in this study can be found in the Supplemental Experimental Procedures.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures and two figures and can be found with this article online at doi:10.1016/j.chembiol.2010.10.013.

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REFERENCES

Boudreau, R.L., McBride, J.L., Martins, I., Shen, S., Xing, Y., Carter, B.J., and Davidson, B.L. (2009). Nonallele-specific silencing of mutant and wild-type huntingtin demonstrates therapeutic efficacy in Huntington's disease mice. Mol. Ther. *17*, 1053–1063.

De Souza, E.B., Cload, S.T., Pendergrast, P.S., and Sah, D.W. (2009). Novel therapeutic modalities to address nondrugable protein interaction targets. Neuropsychopharmacology *34*, 142–158.

DiFiglia, M., Sena-Esteves, M., Chase, K., Sapp, E., Pfister, E., Sass, M., Yoder, J., Reeves, P., Pandey, R.K., Rajeev, K.G., et al. (2007). Therapeutic silencing of mutant huntingtin with siRNA attenuates striatal and cortical neuropathology and behavioral deficits. Proc. Natl. Acad. Sci. USA *104*, 17204–17209.

Drouet, V., Perrin, V., Hassig, R., Dufour, N., Auregan, G., Alves, S., Bonvento, G., Brouillet, E., Luthi-Carter, R., Hantraye, P., and Deglon, N. (2009). Sustained effects of nonallele-specific Huntingtin silencing. Ann. Neurol. *65*, 276–285.

Du, Q., Thonberg, H., Wang, J., Wahlestedt, C., and Liang, Z. (2005). A systematic analysis of the silencing effects of an active siRNA at all single-nucleotide mismatched target sites. Nucleic Acids Res. *33*, 1671–1677.

Duyao, M., Ambrose, C., Myers, R., Novelletto, A., Persichetti, F., Frontali, M., Folstein, S., Ross, C., Franz, M., Abbott, M., et al. (1993). Trinucleotide repeat length instability and age of onset in Huntington's disease. Nat. Genet. *4*, 387–392.

Filipowicz, W., Bhattacharyya, S.N., and Sonenberg, N. (2008). Mechanisms of post-transcriptional regulation by microRNAs: are the answers in sight? Nat. Rev. Genet. *9*, 102–114.

Gagnon, K.T. (2010). HD therapeutics—CHDI fifth annual conference. IDrugs *13*, 219–233.

Gagnon, K.T., Pendergraff, H.M., Deleavey, G.F., Swayze, E.E., Potier, P., Randolph, J., Roesch, E.B., Chattopadhyaya, J., Damha, M.J., Bennet, C.F., et al. (2010). Allele-selective inhibition of mutant huntingtin expression with antisense oligonucleotides targeting the expanded CAG repeat. Biochemistry. in press. Published online October 28, 2010.

Grimson, A., Farh, K.K., Johnston, W.K., Garrett-Engele, P., Lim, L.P., and Bartel, D.P. (2007). MicroRNA targeting specificity in mammals: determinants beyond seed pairing. Mol. Cell *27*, 91–105.

Harper, S.Q., Staber, P.D., He, X., Eliason, S.L., Martins, I.H., Mao, Q., Yang, L., Kotin, R.M., Paulson, H.L., and Davidson, B.L. (2005). RNA interference improves motor and neuropathological abnormalities in a Huntington's disease mouse model. Proc. Natl. Acad. Sci. USA *102*, 5820–5825.

Hu, J., Matsui, M., and Corey, D.R. (2009a). Allele-selective inhibition of mutant huntingtin by peptide nucleic acid-peptide conjugates, locked nucleic acid, and small interfering RNA. Ann. N. Y. Acad. Sci. *1175*, 24–31.

Hu, J., Dodd, D.W., Hudson, R.H., and Corey, D.R. (2009b). Cellular localization and allele-selective inhibition of mutant huntingtin protein by peptide nucleic acid oligomers containing the fluorescent nucleobase [bis-o-(aminoethoxy)phenyl]pyrrolocytosine. Bioorg. Med. Chem. Lett. *19*, 6181–6184.

Hu, J., Matsui, M., Gagnon, K.T., Schwartz, J.C., Gabillet, S., Arar, K., Wu, J., Bezprozvanny, I., and Corey, D.R. (2009c). Allele-specific silencing of mutant huntingtin and ataxin-3 genes by targeting expanded CAG repeats in mRNAs. Nat. Biotechnol. *27*, 478–484.

Knudsen, H., and Nielsen, P.E. (1996). Antisense properties of duplex- and triplex-forming PNAs. Nucleic Acids Res. *24*, 494–500.

Koch, T., Rosenbohm, C., Hanson, H.F., Hansen, B., Straarup, E.M., and Kauppinen, S. (2008). Locked nucleic acid: properties and therapeutic aspects. In Therapeutic Oligonucleotides, J. Kurreck, ed. (Cambridge, UK: RSC Biomolecular Sciences, Royal Society of Chemistry), pp. 103–141.

Kremer, B., Goldberg, P., Andrew, S.E., Theilmann, J., Telenius, H., Zeisler, J., Squitieri, F., Lin, B., Bassett, A., Almqvist, E., et al. (1994). A worldwide study of the Huntington's disease mutation. The sensitivity and specificity of measuring CAG repeats. N. Engl. J. Med. *330*, 1401–1406.

Kurreck, J. (2009). RNA interference: from basic research to therapeutic applications. Angew. Chem. Int. Ed. Engl. *48*, 1378–1398.

Liu, J., Carmell, M.A., Rivas, F.V., Marsden, C.G., Thomson, J.M., Song, J.J., Hammond, S.M., Joshua-Tor, L., and Hannon, G.J. (2004). Argonaute2 is the catalytic engine of mammalian RNAi. Science *305*, 1437–1441.

Lombardi, M.S., Jaspers, L., Spronkmans, C., Gellera, C., Taroni, F., Di Maria, E., Donato, S.D., and Kaemmerer, W.F. (2009). A majority of Huntington's disease patients may be treatable by individualized allele-specific RNA interference. Exp. Neurol. *217*, 312–319.

MacDonald, M.E., Ambrose, C.M., Duyao, M.P., Myers, R.H., Lin, C., Srinidhi, L., Barnes, G., Taylor, S.A., James, M., Groot, N., et al. (1993). A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. Cell *72*, 971–983.

Miller, V.M., Xia, H., Marrs, G.L., Gouvion, C.M., Lee, G., Davidson, B.L., and Paulson, H.L. (2003). Allele-specific silencing of dominant disease genes. Proc. Natl. Acad. Sci. USA *100*, 7195–7200.

Nasir, J., Floresco, S.B., O'Kusky, J.R., Diewert, V.M., Richman, J.M., Zeisler, J., Borowski, A., Marth, J.D., Phillips, A.G., and Hayden, M.R. (1995). Targeted disruption of the Huntington's disease gene results in embryonic lethality and behavioral and morphological changes in heterozygotes. Cell *81*, 811–823.

Omi, K., Hachiya, N.S., Tokunaga, K., and Kaneko, K. (2005). siRNA-mediated inhibition of endogenous Huntington disease gene expression induces an aberrant configuration of the ER network in vitro. Biochem. Biophys. Res. Commun. *338*, 1229–1235.

Pfister, E.L., and Zamore, P.D. (2009). Huntington's disease: silencing a brutal killer. Exp. Neurol. *220*, 226–229.

Pfister, E.L., Kennington, L., Straubhaar, J., Wagh, S., Liu, W., DiFiglia, M., Landwehrmeyer, B., Vonsattel, J.P., Zamore, P.D., and Aronin, N. (2009). Five siRNAs targeting three SNPs may provide therapy for three-quarters of Huntington's disease patients. Curr. Biol. *19*, 774–778.

Reddy, P.H., Williams, M., Charles, V., Garrett, L., Pike-Buchanan, L., Whetsell, W.O., Jr., Miller, G., and Tagle, D.A. (1998). Behavioural abnormalities and selective neuronal loss in HD transgenic mice expressing mutated full-length HD cDNA. Nat. Genet. *20*, 198–202.

Sanchez, I., Mahlke, C., and Yuan, J. (2003). Pivotal role of oligomerization in expanded polyglutamine neurodegenerative disorders. Nature *421*, 373–379.

Schwarz, D.S., Ding, H., Kennington, L., Moore, J.T., Schelter, J., Burchard, J., Linsley, P.S., Aronin, N., Xu, Z., and Zamore, P.D. (2006). Designing siRNA that distinguish between genes that differ by a single nucleotide. PLoS Genet. *2*, e140.

Smith, R.A., Miller, T.M., Yamanaka, K., Monia, B.P., Condon, T.P., Hung, G., Lobsiger, C.S., Ward, C.M., McAlonis-Downes, M., Wei, H., et al. (2006). Antisense oligonucleotide therapy for neurodegenerative disease. J. Clin. Invest. *116*, 2290–2296.

van Bilsen, P.H., Jaspers, L., Lombardi, M.S., Odekerken, J.C., Burright, E.N., and Kaemmerer, W.F. (2008). Identification and allele-specific silencing of the mutant huntingtin allele in Huntington's disease patient-derived fibroblasts. Hum. Gene Ther. *19*, 710–719.

Walker, F.O. (2007). Huntington's disease. Lancet *369*, 218–228.

Wang, Y., Juranek, S., Li, H., Sheng, G., Tuschl, T., and Patel, D.J. (2008). Structure of an argonaute silencing complex with a seed-containing guide DNA and target RNA duplex. Nature *456*, 921–926.

Wheeler, T.M., Sobczak, K., Lueck, J.D., Osborne, R.J., Lin, X., Dirksen, R.T., and Thornton, C.A. (2009). Reversal of RNA dominance by displacement of protein sequestered on triplet repeat RNA. Science *325*, 336–339.

White, J.K., Auerbach, W., Duyao, M.P., Vonsattel, J.P., Gusella, J.F., Joyner, A.L., and MacDonald, M.E. (1997). Huntingtin is required for neurogenesis and is not impaired by the Huntington's disease CAG expansion. Nat. Genet. *17*, 404–410.

Zhang, Y., Engelman, J., and Friedlander, R.M. (2009). Allele-specific silencing of mutant Huntington's disease gene. J. Neurochem. *108*, 82–90.

