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Regular paper

Silencing of genes responsible for polyQ diseases using chemically modified single-stranded siRNAs

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Polyglutamine (polyQ) diseases comprise a group of nine genetic disorders that are caused by the expansion of the CAG triplet repeat, which encodes glutamine, in unrelated single genes. Various oligonucleotide (ON)-based therapeutic approaches have been considered for polyQ diseases. The very attractive CAG repeat-targeting strategy offers selective silencing of the mutant allele by directly targeting the mutation site. CAG repeat-targeting miRNA-like siRNAs have been shown to specifically inhibit the mutant gene expression, and their characteristic feature is the formation of mismatches in their interactions with the target site. Here, we designed novel single-stranded siRNAs that contain base substitutions and chemical modifications, in order to develop improved therapeutic tools with universal properties for several polyQ diseases. We tested these ONs in cellular models of Huntington's disease (HD), spinocerebellar ataxia type 3 (SCA3) and dentatorubral-pallidoluysian atrophy (DRPLA). Selected siRNAs caused the efficient and selective downregulation of the mutant huntingtin, ataxin-3 and atrophin-1 levels in cultured human fibroblasts. We also prove the efficiency of novel ONs, with chemical modification pattern mainly containing 2'-fluoro (2'F), in HD mouse striatal cells.

Key words: siRNA, CAG repeats, polyglutamine diseases, Huntington's disease

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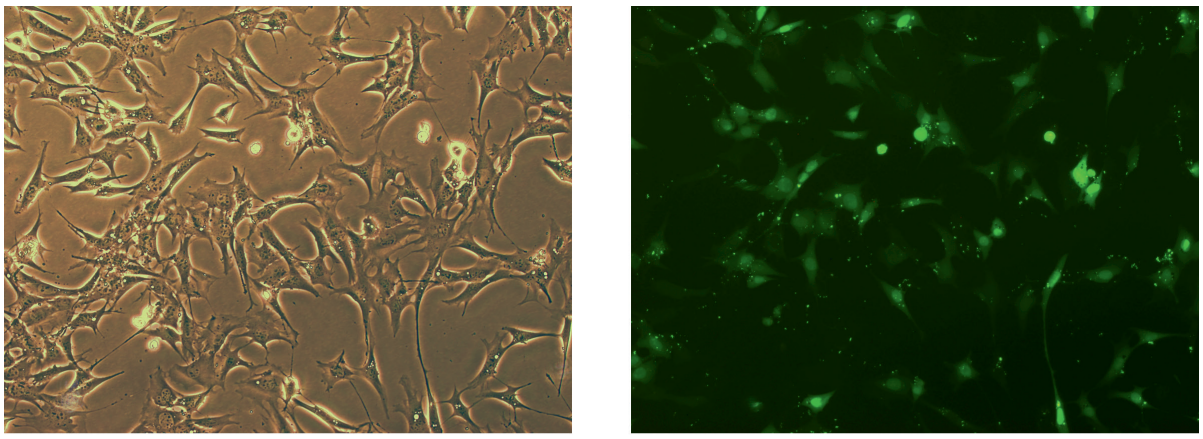
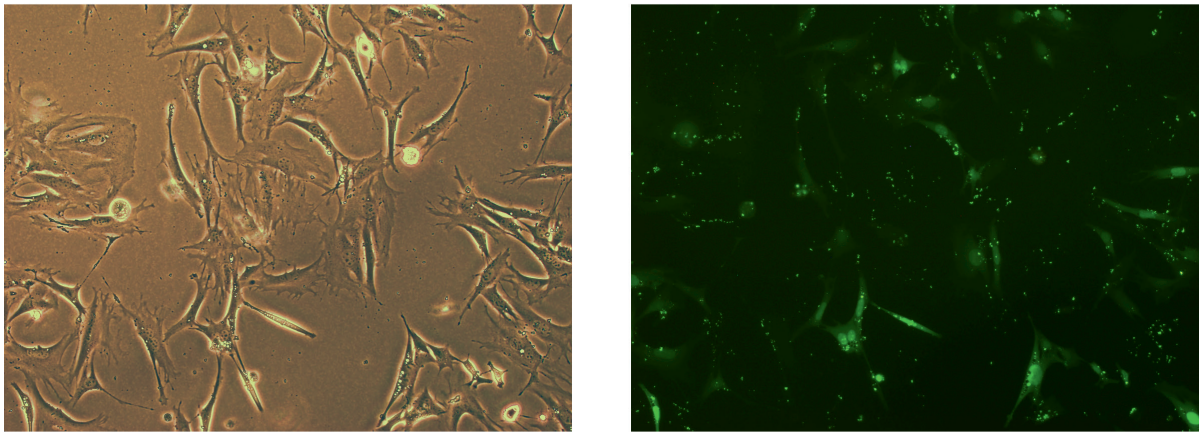
Abbreviations: DRPLA (dentatorubral-pallidoluysian atrophy), HD (Huntington's disease), ONs (oligonucleotides), polyQ (polyglutamine), sd-siRNAs (self-duplexing siRNAs), PS (phosphothioate modification), RISC (RNA-induced silencing complex), SCA3 (spinocerebellar ataxia type 3), ss-siRNAs (single-stranded siRNAs), 2'OMe (2'-O-methyl modification), 2'F (2'-fluoro modification)



ST*Hdh* 7/7 BlockIT siRNA



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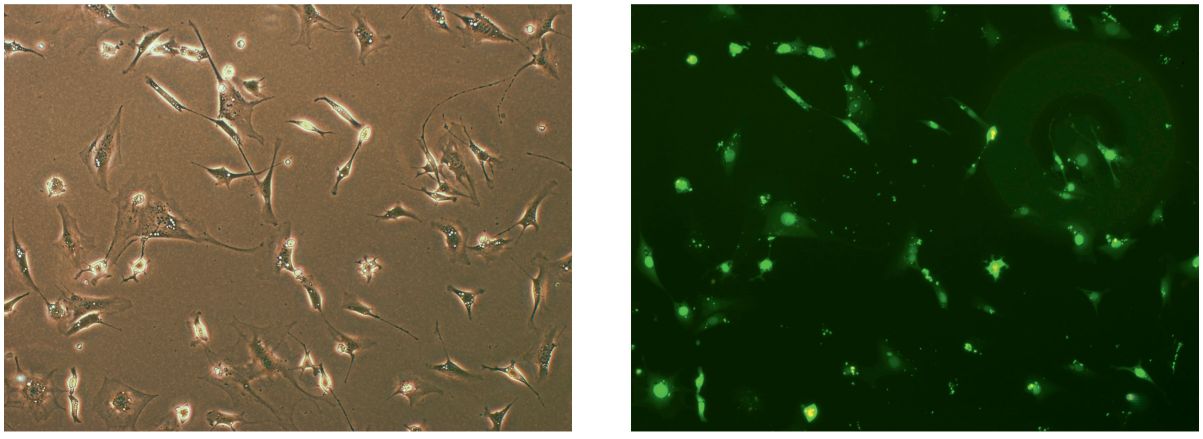

ST*Hdh* 111/111 BlockIT siRNA

Supplementary Fig. 1. Transfection efficiency for ST*Hdh* cell lines assessed 24 h after transfection with 25 nM fluorescently labeled siRNA BlockIT. Repeat tract lengths in *Htt* are indicated for the three analyzed cell lines.