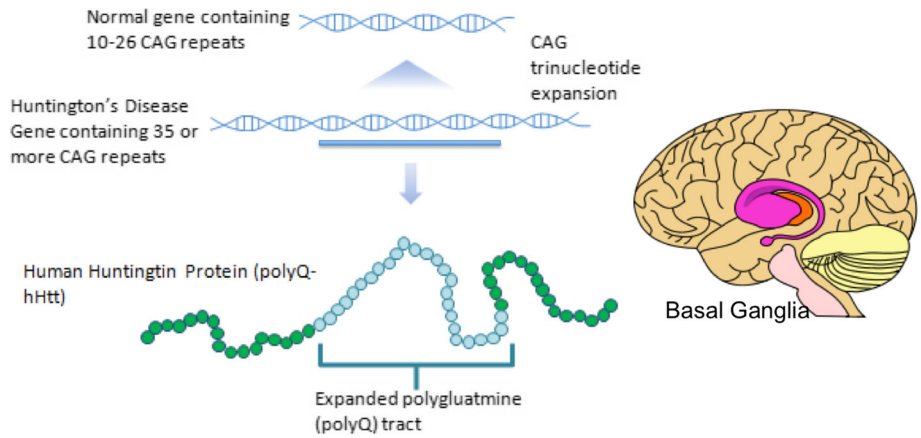


**Fig. 1** Huntingtin mutation and basal ganglia. Huntington's results from an expanded polyglutamine tract, encoded by CAG repeats. The basal ganglia is the most severely affected region in HD

increased excitotoxicity, glial activation, and an increase in astrocytes [6], as well as altered an epigenetic pattern [7]. HTT may interfere with intercellular interactions like trophic support and can be ameliorated with ectopic BDNF [8]. Mutant HTT inhibits axonal transport of BDNF-containing vesicles in corticostriatal neurons [9].

It is not well understood why, but HTT affects certain cell types selectively — particularly neurons of the basal ganglia and especially striatal medium spiny neurons where cytotoxicity manifests earliest. Other affected areas include the substantia nigra and layers 3, 5, and 6 of the cerebral cortex [10].

## Therapeutic strategies

Many HD therapeutics target downstream consequences and symptoms of the causal pathogenic mutation in the Huntingtin (HTT) gene, while a few next-generation therapies target mHTT itself (see Table 1) [11]. The only approved drug is tetrabenzene, which palliates motor abnormalities. There are no disease-modifying therapies currently available to patients. It is known that conditional silencing of transgenic mutant HTT (mHTT) reverses HD in mice, showing that mHTT is required for HD progression [12].

Small molecule interventions for HD are an exciting (albeit most palliative) area of research, but beyond the scope of this review [13–20]. Targeting the primary cause of HD has become possible in recent years due to advancements in RNA interference by small noncoding RNAs (sncRNAs), such as synthetic siRNA and shRNA [21]. Despite the variations in RNAi constructs, all act by binding the mRNA of a target gene to either block translation or cause degradation of the transcript Fig. 2.

shRNA is a synthetic RNA molecule with a *s*hort *h*airpin secondary structure. Because it is delivered on a DNA plasmid rather than as double stranded RNA (e.g., siRNA), shRNA can be continually expressed for months or years.

After transcription, the product mimics pri-microRNA and is processed by Drosha to create pre-shRNA that is exported from the nucleus by Exportin 5. Then the pre-shRNA is processed by Dicer and binds to RISC complex. The passenger strand is degraded, and the antisense guide strand directs RISC to degrade complementary target mRNA (such as HTT).

Most HD RNAi therapies to date have been based on synthetic siRNAs or antisense oligonucleotides (ASOs) delivered naked, conjugated to cholesterol, or with lipofectamine. Unfortunately, these drugs require repeated dosing, commonly exhibit off target effects, and exert renal and hepatic toxicity [21] Fig. 3.

## Off-target effects (OTEs): shRNA vs siRNA

The off-target accuracy of shRNA versus siRNA is an open question. A few studies have indicated that siRNA has more off-target effects (OTEs) than shRNA when compared head-to-head.

Mehaffey et al. [22] treated HCT-116 carcinoma cells with either an siRNA duplex or an inducible shRNA of the same core sequence, targeting the TP53 gene, and analyzed gene expression changes 24 h post-treatment via microarray hybridization. They found a substantially higher proportion of off-target genes upregulated or downregulated in cells treated with siRNA rather than shRNA. The degree of on-target knockdown was comparable.

As a follow up to this study, Klinghoffer et al. [23] at Merck & Co repeated this experiment and included additional mRNA targets: CDKN1A, E2F1, EZH2, FDXR. The shRNA was compared to siRNA at various concentrations, and the authors also used a cell line stably expressing their constructs to control for differential transfection efficiencies. The results confirmed the prior work, showing both