Effect of Small Molecule Splicing Modulators on proteome and transcriptome of HD stem cell derived neurons

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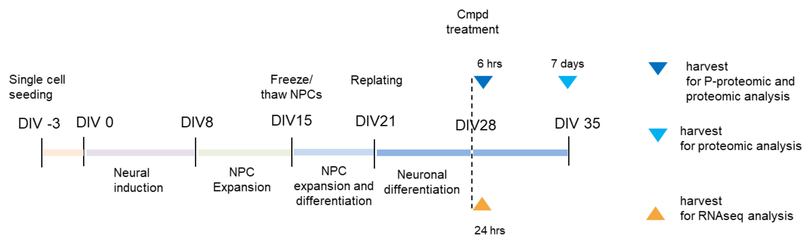
Introduction

Small molecules which cause alternative Huntingtin (HTT) pre-mRNA splicing and result in HTT protein lowering have been developed as therapeutics for HD. Preclinical studies in BACHD mice confirmed *in vivo* splicing activities on HTT at RNA level, and subsequently HTT protein reduction. However, proliferating cell toxicity is observed at higher doses and cell cycle blockade is observed in cell models. As yet, the detailed mechanism for both splicing efficacy and toxicity remain unclear

Here, to investigate such mechanisms, we performed a study comparing the effects of two splicing modulators (Branaplam and SMSM1) as well a non-RNA HTT lowering compound (Deoxygedunin), using transcriptomics, proteomics and phospho-proteomics analysis of compound treated HD stem cell derived cortical neurons.

Abbreviations NPC: Neural Progenitor Cells, FC: fold-change

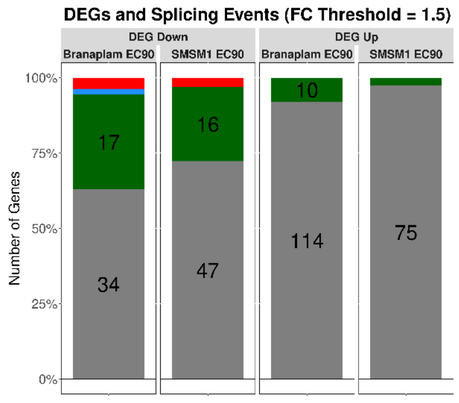
Study Design



Two HD heterozygous pluripotent stem cell lines, expressing mutant HTT (56CAG and 72CAG), were differentiated into forebrain cortical neurons by the method described in the schematic above.

Following differentiation, neurons were incubated with compounds on day 28 of in vitro differentiation (DIV) and harvested for phospho-proteomic and proteomic analysis (72CAG neurons) and for RNAseq analysis (56CAG neurons) after the indicated duration of compound treatment.

Differential splicing events



Comparison of SMSM1 and Branaplam

For both splicing modulators more regulations associated with NMDs/j and D/UJ can be observed for down-regulated events.

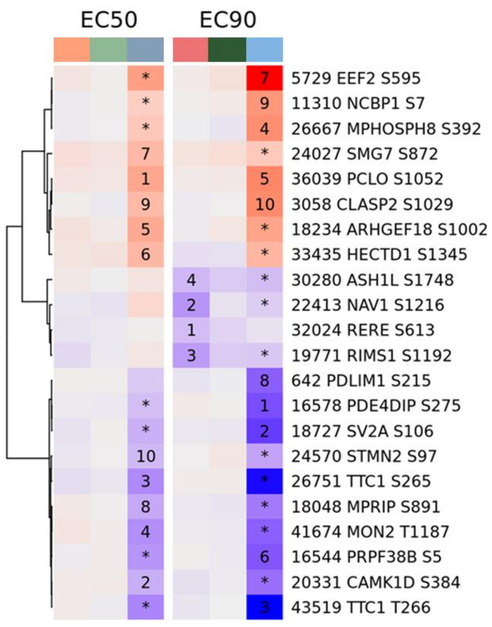
DEG + NMSs/i + DIJ
DEG + NMSs/i
DEG + DIJ
DEG

Differentially Expressed Genes (DEG) determined using DESeq2 (|FC| >= 1.5, Adj. P-value < 0.05)

Differentially Utilized Exon Junction (DUJ) determined using DEXSeq (|FC| >= 1.5, Adj. P-value < 0.05),

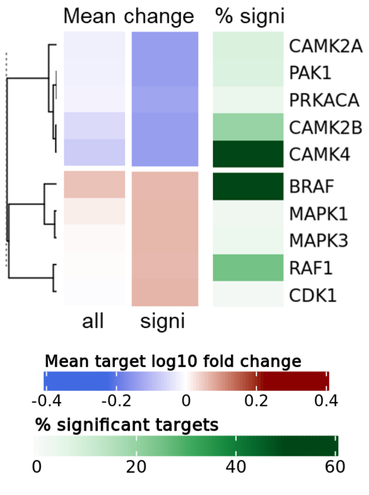
Significant isoform switch effecting annotated NMD (NMDs/i) isoform switch determined using IsoformSwitchAnalyzer (|Delta Isoform Fraction (dIF)| > 0.1, FDR < 0.05)

Analysis of Phosphoproteomics Data



Regulated Phospho-sites

Heatmaps show the top 10 regulated (up/down) phospho-sites for different compounds according to the adjusted p-value. Small numbers give ranking in Top 10 and asterisks indicate additional significant proteins.

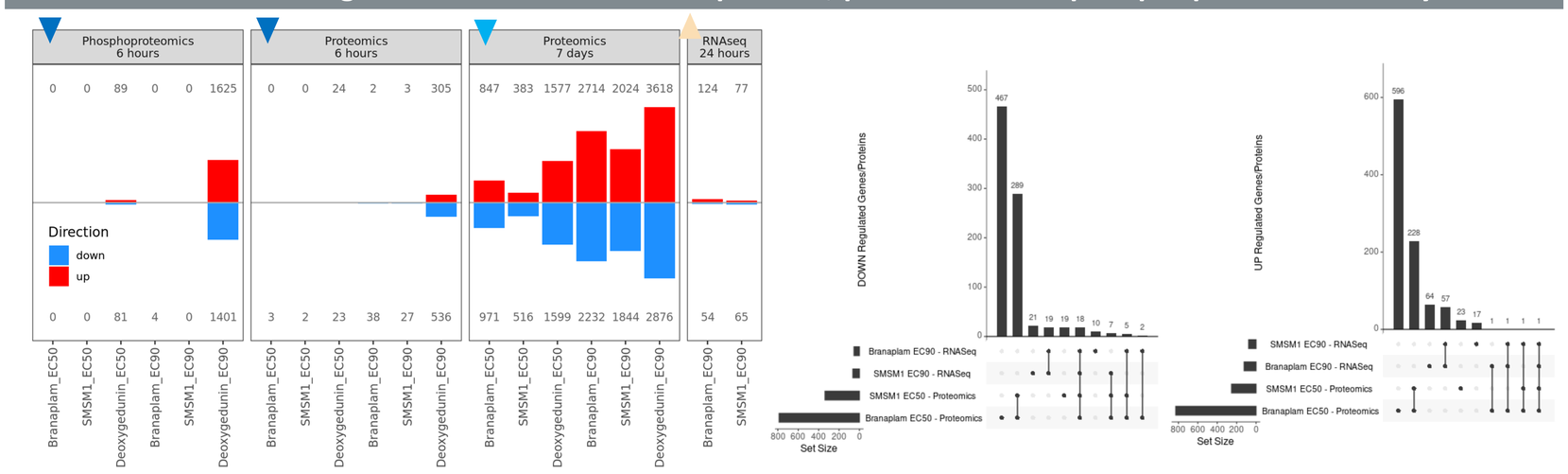


Kinase target enrichment analysis

We performed a kinase target enrichment analysis for Deoxygedunin.

Heatmaps show (from left to right) average log10 phosphorylation FC over all measured target sites of a kinase, mean log10 phosphorylation FC over significantly regulated target sites of a kinase and the fraction of annotated target sites per kinase that exhibit a significant regulation.

Overview of regulated features in transcriptomics, proteomics and phosphoproteomics study



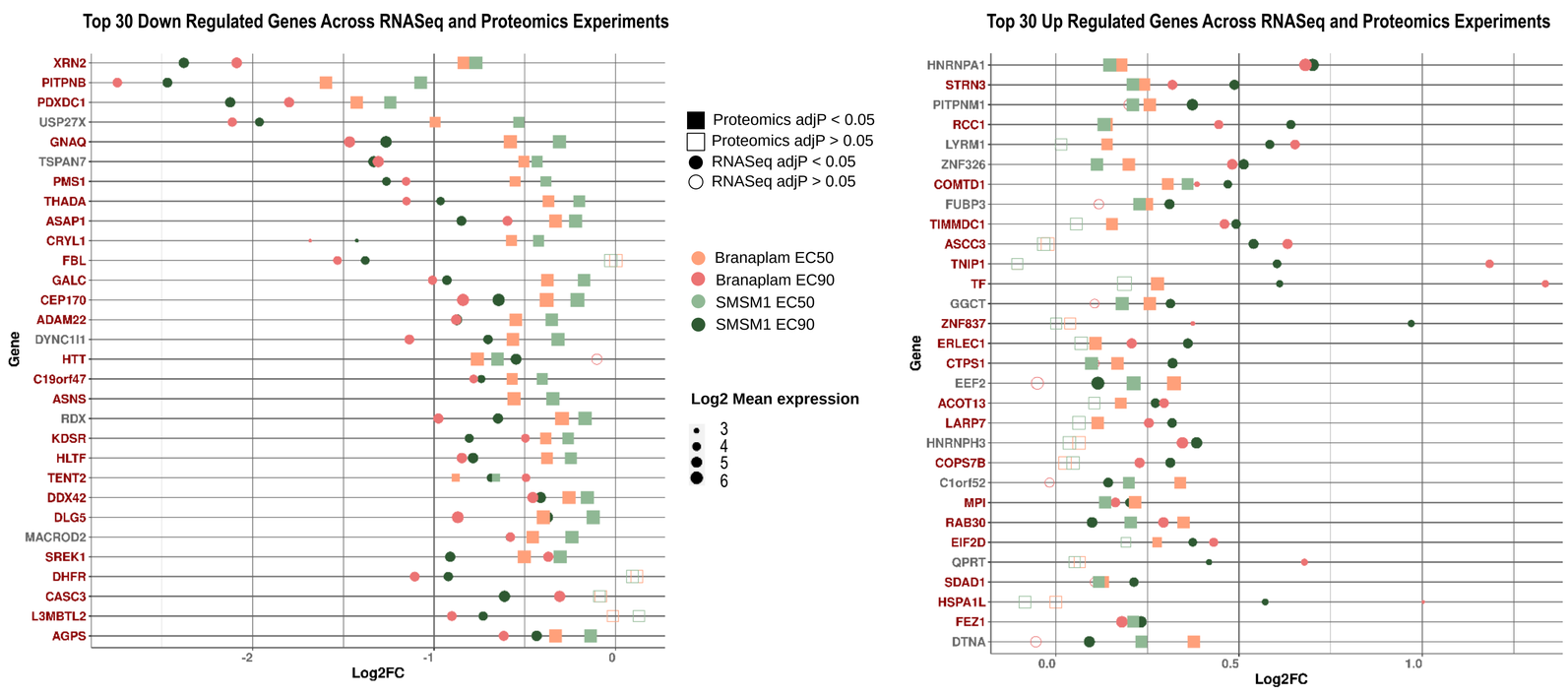
Significantly regulated protein groups and genes

Here, we show the number of up- (red) and down- (blue) regulated features for all time points and concentrations for Branaplam, SMSM1 and Deoxygedunin (proteomics only). The number of regulations is increased for both higher concentrations and later time points. Branaplam and SMSM1 show very similar numbers of regulations.

Overlap of regulated genes between proteomics and transcriptomics

The highest overlap of regulations can be observed between SMSM1 and Branaplam within the same omics type. In addition, a subset of 18 genes is consistently down-regulated across all conditions whereas only a few individual genes appear to be up-regulated consistently across all groups.

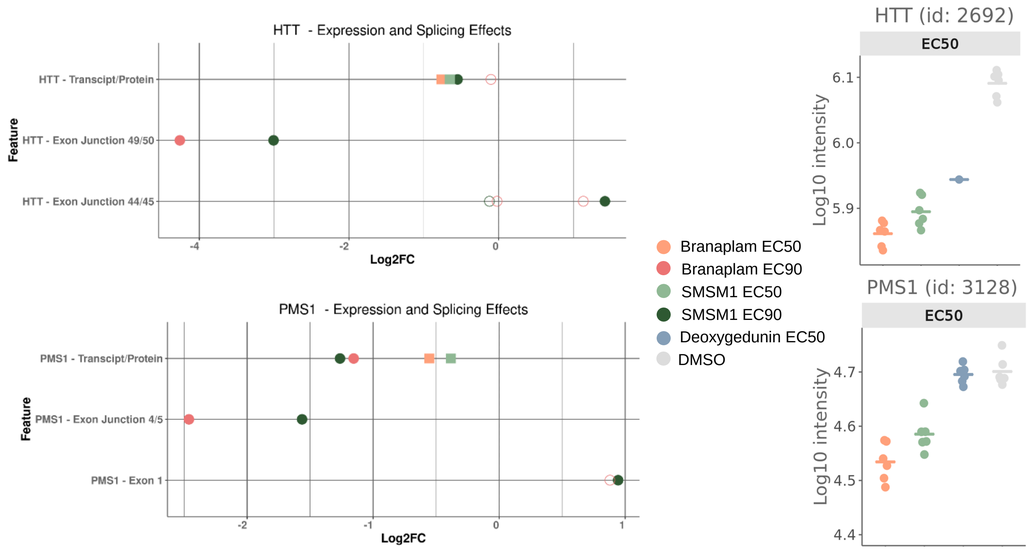
Comparison of proteomics and transcriptomics regulations



Top 30 up- and down-regulated genes shared between RNASeq and Proteomics

To compare changes in transcriptomics (circle) and proteomics (square), the observed log2 FC values for Branaplam and SMSM1 are displayed side by side for the most significant 30 up- and down-regulated genes. Gene symbols associated with a significant splicing event (differential exon or exon junction utilization) are marked in red. These events occur more often for the down-regulated genes like XRN2, PITPNB, PMS1 or HTT.

Detail plots for HTT and PMS1



Expression and Splicing effects for HTT

Protein expression as well as Exon Junction 49/50 are down-regulated for both Branaplam and SMSM1 at EC50. In contrast, Exon Junction 44/45 is not regulated or even significantly up-regulated (SMSM1 EC90).

Expression and Splicing effects for PMS1

For PMS1 a reduction of protein expression can be observed for the splicing modulators Branaplam and SMSM1 but not for Deoxygedunin.

Conclusions

- Parallel transcriptomics, proteomics & phosphoproteomics analysis of compound treated HD neurons revealed common and compound specific signatures for two HTT lowering splicing modulators, distinct from the profile obtained by Deoxygedunin which lowers HTT by a non-RNA based MOA
- Analysis of differential splicing events revealed a higher number of events for down-regulated genes. When comparing the top 30 down-regulated genes to their protein expression levels, a high consistency in the regulation direction was observed. Proteomic changes also identified further indirect changes not linked directly to splicing events.
- Phospho-proteomic data at the 6 hr treatment time point showed surprisingly few changes for the splicing modulators and several for Deoxygedunin suggesting an indirect kinase cascade regulation of HTT lowering. Deoxygedunin and close analog gedunin have two proposed MOAs (TrkB agonist and Hsp90-co-chaperone disruptor). This data does not directly support or exclude either MOA.
- From an HD therapeutic target perspective, we were intrigued to observe a direct splicing event mediated protein lowering effect on PMS1 (aka MLH2) for both Branaplam and SMSM1. PMS1 is a component of the post-replicative DNA mismatch repair system (MMR) identified by GWAS analysis of HD patients. The impact of PMS1 lowering via compound treatment on somatic instability will be investigated
- PMS1 is also involved in DNA damage signaling, a process which induces cell cycle arrest and can lead to apoptosis in case of major DNA damages, so could be implicated in proliferating cell arrest observed with splicing modulators
- Further analysis of the data is ongoing and will be shared via HDinHD and publication in due course