Phosphoproteomic analysis of TrkB receptor activation by a novel monoclonal antibody agonist: Implications for the treatment of Huntington's disease.

Barbara Kracher[1], Jan-Philip Schülke[1], Nikisha Carty[2], Britta Huscher[2], Annette Gärtner[2], Galina Bursova[2], Tom Schwagarus[2], Liesbeth Micholt[2], Elizabeth van der Kam[2], Jonathan Bard[3], Jim Rosinsk3, Ignacio Munoz-Sanjuan[3], Vahri Beaumont[3]

[1] Evotec Munich GmbH, Am Klopferspitz 19a, 82152 Planegg/Martinsried, Germany
[2] Evotec SE, Essener Bogen 7, 22419 Hamburg, Germany (Corp. HQ)
[4] CHDI Management/CHDI Foundation, 6080 Center Drive, Suite 700, Los Angeles, CA 90045, USA

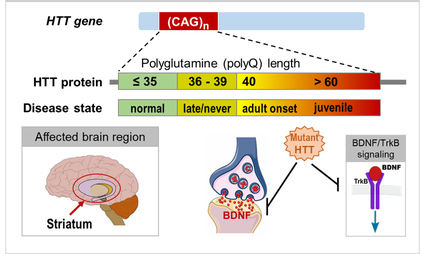
Contact: Barbara.Kracher@evotec.com

Introduction

Huntington's disease (HD) is a neurodegenerative disorder caused by CAG expansions in the *huntingtin* gene (*HTT*).

Alterations of the neurotrophin tyrosine receptor kinase (TrkB) signaling pathway can contribute to HD pathophysiology, as activation of TrkB by brain-derived neurotrophic factor (BDNF) is crucial for the survival, differentiation and synaptic plasticity of striatal neurons.

Huntington's disease (HD)



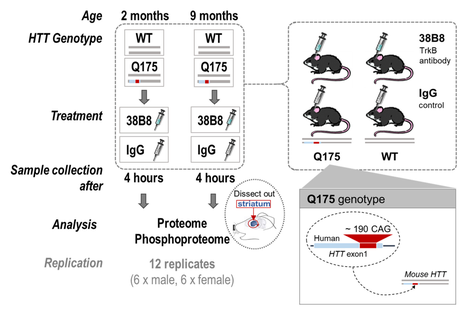
A reduction of BDNF in the striatum, cortex and hippocampus from HD post-mortem brain tissue and reduced cortico-striatal BDNF trafficking in HD mouse models has been described, whilst other reports have demonstrated normal levels of BDNF but impaired downstream TrkB receptor signaling.

Here we investigated a potential therapeutic approach to reverse deficits in HD through activation of BDNF/TrkB signaling in an HD mouse model using the novel TrkB agonistic mouse monoclonal antibody 38B8[1)].

Methods

We monitored the effects of the TrkB agonistic antibody 38B8, 4 hours after bilateral intrastriatal injection, on the proteome and phosphoproteome of wild type (WT) and zQ175DN heterozygous (Q175) mice at 2 months (presymptomatic) and 9 months of age (symptomatic).

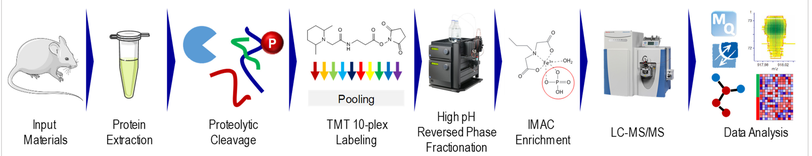
Study design



Experimental workflow

- Protein extraction and digestion with Trypsin/LysC
- Tandem mass tag (TMT) labeling of peptides and sample pooling
- Fractionation by high pH reversed-phase chromatography (10 fractions)
- Phospho-peptide enrichment by metal affinity chromatography (IMAC)
- Separate LC MS/MS analyses on a Q-Exactive HF mass spectrometer
- MaxQuant[2)] analysis of RAW files (using the Andromeda search engine)
- Bioinformatic data analysis in R[3)]

TMT 10-plex PhosphoScout®

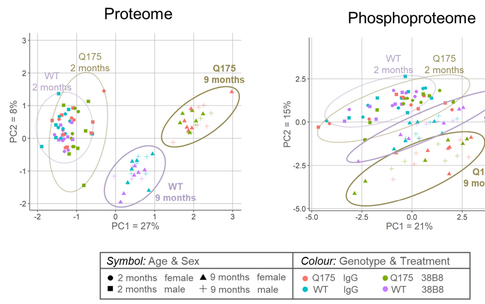


Bioinformatic data analysis

- TMT reporter intensities were normalized to the median protein/site intensity per TMT 10-plex.
- Changes of protein expression and phosphorylation were analyzed using the limma[4)] package for linear modeling and statistical testing (moderated t-tests).
- Gene Ontology enrichment was analyzed using GOstats[5)] (conditional hypergeometric test).
- P values were corrected for multiple hypothesis testing using the Benjamini-Hochberg procedure.

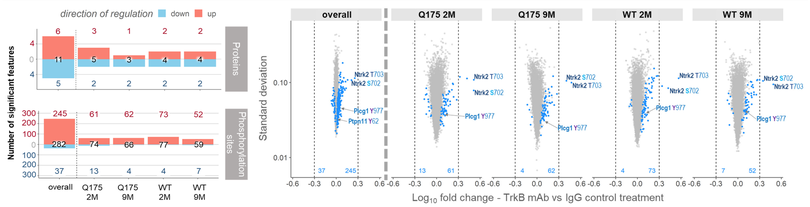
Statistical and Bioinformatic Analysis

Quality Control and Global Analysis



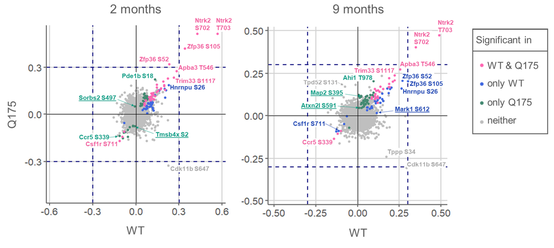
- In the principal component analysis (PCA) the samples clustered mainly by age, (9 months vs. 2 months).
- The 9 months samples, but not at 2 months samples, further clustered by genotype, (Q175 vs. WT).
- No separation of samples detectable according to the treatment (38B8 vs. IgG)

Identification of protein expression and phosphorylation changes



While at both ages global striatal protein expression levels were largely unaffected 4 hours after 38B8 injection, we found over 200 significantly regulated phosphorylation sites, mostly exhibiting increased phosphorylation.

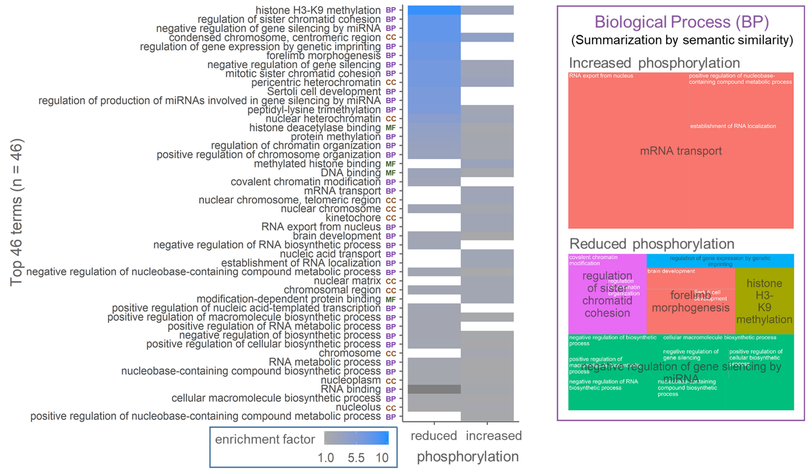
Comparison of 38B8 response between WT and Q175 genotypes



The observed changes in protein phosphorylation upon 38B8 treatment were similar between WT and Q175 (HD) mice and no significant differences were detected between the two genotypes.

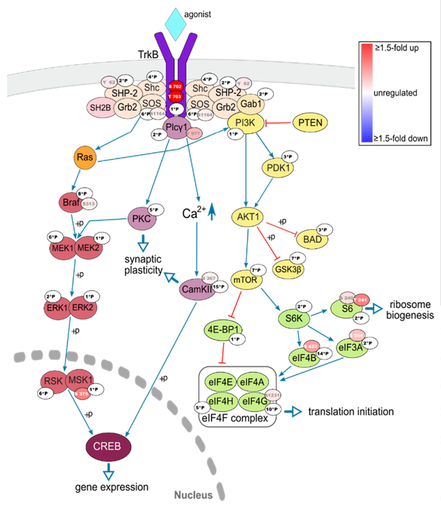
Gene Ontology enrichment analysis

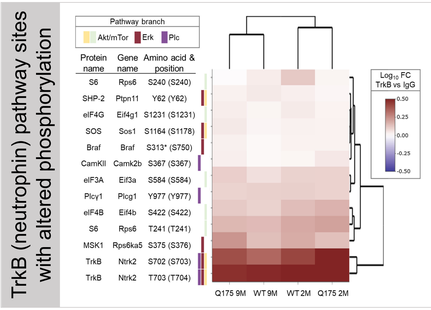
Functional enrichment analyses, based on gene ontology (GO) terms, revealed that proteins with regulated phosphorylation sites are mostly involved in regulation of gene expression, more specifically in mRNA transport, ribosome biogenesis and translation initiation.



Key findings

Treatment with 38B8 activates TrkB signaling in WT and Q175 (HD) mice

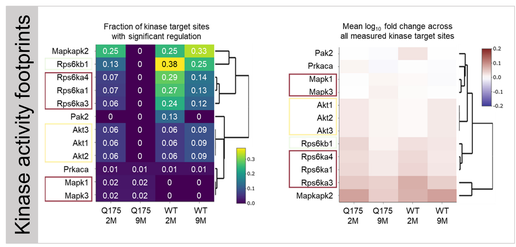




Sites with significantly increased phosphorylation after 38B8 injection were found on components of all three branches of the BDNF/TrkB (neutrophin) signaling pathway and the 38B8 response was similar in WT and Q175 mice of both ages.

Thus, our data provides evidence for activation of all three pathway branches in both wild-type and Q175 (HD) mice.

Erk1/2 and Akt1/2/3 are key mediators of the 38B8 response

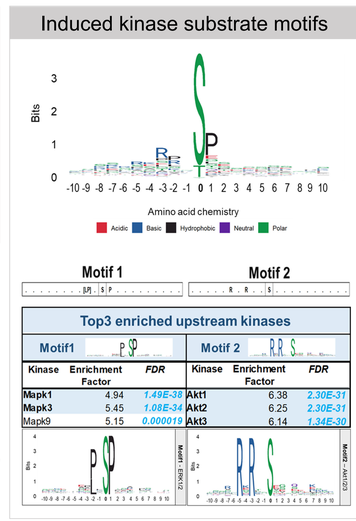


Analysis of kinase activity footprints, computed from the phosphoproteome data based on (site-specific) kinase-target interactions annotated in the SIGNOR[4)] database, and of kinase substrate motifs, identified by the Motif Description Length (MoDL)[5)] algorithm, indicate a key role for the kinases Erk1/2 and Akt1/2/3 in meditating the response to the 38B8 treatment.

*Sequence logos were generated from aligned sequences of all (top) significantly up-regulated sites (**overall** comparison) or subsets of these sequences containing the indicated motif.*

Significance of upstream kinase enrichment was determined using a Fisher exact test.

Induced kinase substrate motifs



Conclusions

- In this study, we monitored global phosphoproteome and proteome changes after bilateral injection of a TrkB agonistic antibody, 38B8, into the striatum of wild-type and Q175 (HD) mice.
- We could trace the 38B8 response from TrkB receptor activation (direct phosphorylation) through the subsequent signaling cascade via Plcγ1 (direct phosphorylation), Erk1/2 and Akt1/2/3 (kinase activity and identification of regulated motifs).
- Similar levels of pathway activation were observed in wild-type and Q175 mice, indicating that the agonist function is retained in HD mice.
- Taken together, our findings demonstrate the functional activity of 38B8 in an HD mouse model *in vivo*, which suggests that direct TrkB receptor activation could be a viable approach for the treatment of HD.

References

1) Todd D et al., *PLOS One* (2014) 9(2):e87923
2) Cox J & Mann M, *Nat Biotechnol* (2008) 26:1367-1372
3) R Core Team, *R Foundation for Statistical Computing* (2018)
4) Ritchie MA et al., *Nucleic Acids Res* (2015) 43(7):e47
5) Falcon S & Gentleman R, *Bioinformatics* (2007) 23(2):257-258
6) Perfetto L et al., *Nucleic Acids Res* (2015) 44(D1):D548-D554
7) Ritz A et al., *Bioinformatics* (2009) 25(1):14-21

Acknowledgements

This work was funded by CHDI Foundation.