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Inhibition of specific histone deacetylases suppresses pathogenesis in a *Drosophila* model of Huntington's disease

PhD Thesis

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INTRODUCTION

Huntington's disease (HD) is a late-onset neurodegenerative disease that affects primarily the striatum, and results in progressive motor disturbances as well as cognitive and psychological impairments. It belongs to a family of disorders that are caused by expanded CAG triplet repeat sequences which encode expanded polyglutamine (polyQ) repeats in the affected protein. This mutant form of the protein interacts aberrantly with itself, resulting in the accumulation of various forms of oligomers and aggregates, which might by themselves be toxic to the cell; it also interacts abnormally with a number of cellular proteins, leading to disturbed homeostasis, neuronal dysfunction, and death.

Transcriptional regulators, such as the histone acetyltransferases or methyltransferases, are an example of proteins with which the mutant Huntingtin protein interacts abnormally, resulting in altered nucleosomal dynamics and dysregulation of transcription. Chemical agents that restore the acetylation balance at least partially have shown great promise in ameliorating the effects of the mutant polyQ protein in several model systems, and some histone deacetylase (HDAC) inhibitors are currently in human clinical trials. The studies described here seek to bring a better understanding of the role of histone deacetylases in HD pathology.

Since HD is monogenetic, the disease pathogenesis can be readily studied in model organisms, one of which is *Drosophila melanogaster*. Our laboratory has generated a fly model of HD that recapitulates many aspects of the human disease, including the appearance of intracellular inclusions, as well as progressive neurodegeneration, motor dysfunction, sleep disturbances and early death. We took advantage of the large array of genetic tools and

assays available in *Drosophila* to investigate these disease phenotypes in response to altered histone deacetylase levels. We found that partial inhibition of select HDAC activities increases the eclosion rate, reduces photoreceptor neuron cell loss, and improves motor performance of mutant Htt expressing flies, without altering inclusion body formation. Reducing sirtuin levels also rescues the sleep abnormalities observed in *Httex1p Q93* flies. We demonstrate that feeding flies the HDAC inhibitor butyrate corrects hypoacetylation of the K27 lysine of histone H3, and in a proof of principle study, we show that manipulating HDAC activities can restore the aberrant transcriptional output of a small set of dysregulated genes.

Our results suggest that therapeutic strategies designed to target Rpd3, Sir2 and Sirt2 activities specifically and administered in combination, are likely to result in improved neuronal benefit with no reduction in longevity, which could translate into a promising therapeutic means for the treatment of this devastating disease.

AIMS

1. Develop new techniques to measure mutant Htt induced degeneration in order to validate the finding that broad spectrum class I-II histone deacetylase (HDAC) inhibitors reduce photoreceptor neuron degeneration of *elav>Httex1p Q93* flies.

2. Test the effect of sirtuin inhibitors on mutant Htt induced pathology.

3. Using mutant alleles for each HDAC alone and in combination, determine which histone deacetylases are responsible for the effect seen with the HDAC inhibitor treatment.

4. Investigate if manipulating HDAC levels can expand the shortened life span of *Httex1p Q93* flies.

5. Evaluate the circadian rhythm of mutant Htt expressing flies and test if the sleep abnormalities can be restored by lowering Sir2 activity.

6. Test if broad spectrum histone deacetylase inhibitor treatment alters the aggregation dynamics of the mutant Huntingtin protein.

7. Determine if global histone acetylation levels are altered in *Httex1p Q93* flies and if it can be restored by HDAC inhibitor treatment.

8. Establish a set of genes to study transcriptional dysregulation of mutant Htt expressing flies and evaluate the effect of HDAC mutants on these genes.

MATERIALS AND METHODS

- Compound feeding and genetic interaction tests using flies expressing a *UAS-Httex1p Q93* transgene
- Generation of RNA interference lines
- Assessment of the neuronal degeneration using the pseudopupil technique
- Climbing assay
- Pigment measurement of the compound eye
- Evaluation of circadian activity using TriKinetics activity monitors
- Confocal analysis of larval brain samples
- Western analysis of adult head tissue
- Measurement of gene expression levels using quantitative real time PCR
- Statistical analysis

RESULTS

1. We have previously shown that the broad spectrum histone deacetylase inhibitors butyrate and SAHA reduce photoreceptor neuron loss of *Httex1p Q93* flies. To validate this finding in independent assays, we measured the progressive motor dysfunction of *Httex1p Q93* flies in a climbing assay and a nine-choice geotaxis assay and found that feeding butyrate improves the motor function of these flies. Expressing the *Htt* transgene with an eye specific driver instead of the pan-neuronal driver used in most of our experiments, we showed that the eye pigmentation of *Httex1p Q93* flies diminishes as they age, which is suggestive of a progressive loss of the pigment cells; butyrate treatment can counteract this effect.

2. Of the ten histone deacetylase enzymes in the fly, five belong the NAD^+-dependent class of sirtuins, which butyric acid and SAHA have no effect on. To determine if this class of HDACs also has impact on mutant Htt induced pathology, we fed the flies chemical inhibitors of sirtuins, including niacin, nicotinamide, and several Sirt2 specific compounds, and found that they all reduce photoreceptor neuron degeneration of *Httex1p Q93* flies.

3. Most drugs target several different proteins, therefore the reduction of neurodegeneration seen with chemical inhibitors could either be attributed to small incremental contributions of all histone deacetylase enzymes or it could be the result of primarily one or a few HDACs. To distinguish between these possibilities, we turned to genetic tools and tested multiple alleles (some of which we generated) for each of the 10 deacetylases of the fly. We found that *Httex1p Q93* induced pathology is most sensitive to levels of three

HDACs: Rpd3, Sir2 and Sirt2, as partial reduction of only these reduces the photoreceptor neuron loss of mutant Htt expressing flies. We also found that combinatorial reduction of any two of these improves the outcome over single mutants, suggesting that the deacetylases target distinct pathways.

4. Expressing the mutant Htt peptide reduces the life span of the flies significantly, and histone deacetylases are known to regulate longevity. Therefore we wanted to know if the neuroprotection achieved by partial reduction of deacetylase activities translates into increased life span. We found that this is not the case; only Rpd3 loss increased the life span of *Httex1p Q93* flies and the change was minor. In contrast, increasing sirtuin activity by overexpressing *Sir2* resulted in a significant increase in life span, but the extent of the neurodegeneration in these flies is unchanged compared to their siblings with normal levels of Sir2. These studies suggest that the molecular mechanisms which increase life span and health span are distinct.

5. One of the consequences of Huntington's disease is a disruption of normal sleep patterns in patients. Studies show that the histone deacetylase activity of SIRT1/Sir2 is regulated in a circadian matter and that the protein itself is recruited to promoters of several core clock genes; although it remains unknown whether manipulation of Sir2 activity alone is sufficient to affect sleep/wake cycles in living animals. We monitored the activity pattern of wild type and *Httex1p Q93* expressing flies with normal or reduced Sir2 levels, and found that mutant Htt expressing flies exhibit various sleep disturbances, including fragmented sleep comprised of many short sleep segments and latency to fall asleep at night. We also found that partial loss of Sir2 corrects these anomalies in *Httex1p Q93* flies. Interestingly, the *Sir2* mutation does not affect the daytime disturbances, suggesting that the role of Sir2 in regulating day and night time activity patterns may be genetically and

pharmacologically separable.

6. One of the hallmarks of Huntington's disease is the appearance of large intracellular inclusions comprised of the mutant and wild type forms of Htt itself as well as various cellular proteins, but role of these aggregates in disease pathology is not well understood. We analyzed the aggregation load in the brains of mutant *Httex1p* expressing larvae fed butyric acid, and found that it does not differ significantly from siblings not treated with the histone deacetylase inhibitor, suggesting that the neuroprotection seen with this drug is not due to its effects on inclusion body formation.

7. The transcriptional output of genes is largely regulated by posttranslational modifications of histones. The mutant Huntingtin protein aberrantly interacts with several histone acetyltransferases, as well as other histone modifying enzymes, which can lead to a disturbed nucleosomal homeostasis. We show that the K27 lysine of histone H3 is indeed hypoacetylated in *Httex1p Q93* flies. Feeding flies butyric acid restores the wild type acetylation level, suggesting that the positive effects of this drug on neurodegeneration are at least in part due to its correcting the acetylation state of histones, and therefore the transcriptional output of dysregulated genes.

8. To demonstrate that manipulating HDAC activities can indeed correct transcriptional dysregulation of *Httex1p Q93* flies, we first needed to establish a set of genes with abnormal mRNA levels, which we did by performing quantitative RT-PCR on candidate genes that have been identified using the results of an earlier microarray experiment. We then evaluated the levels of these mRNAs in mutant Htt expressing flies also carrying a loss of function allele of *Rpd3*, *Sir2* or *Sirt2*, and found that reducing HDAC activities can correct the transcriptional output of some of the genes.

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