Ph.D. Thesis

Investigation into the effects of epigenetic modifications and circadian rhythm defects in a *Drosophila* model of Huntington's disease

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Szeged
2021

Introduction

Epigenetic research performed in a wide variety of pathological conditions during the last decades led to the realization that transcriptional dysregulation resulting from disturbed homeostasis of epigenetic modifications is a common feature of several diseases. One example is Huntington's disease (HD), an autosomal, dominantly inherited, incurable neurodegenerative disorder. HD is characterized by choreiform abnormal involuntary movements, cognitive, behavioral and psychiatric symptoms. Huntington's disease is caused by the abnormal expansion of a polyglutamine coding CAG repeat in the *Huntingtin* gene that contributes to the intracellular aggregation of the toxic, mutant Huntingtin protein (mHtt). One of the main causes of neurodegeneration is transcriptional dysregulation caused by the inhibition of CBP and Gcn5 histone acetyltransferase enzymes through abnormal interactions with the mHtt protein. Neurodegeneration can be alleviated by the overexpression of CBP or by inhibiting the antagonistic histone deacetylase enzymes. Thus, histone acetylation might be a therapeutic target in the treatment of Huntington's disease, however, the key target lysine residues in HD are yet to be identified.

Furthermore, similarly to several other neurodegenerative diseases, circadian rhythm defects are a symptom of Huntington's disease as well. These are also in connection with the CBP histone acetyltransferase that directly interacts with the circadian clock

regulator CLK/ CYC transcription factors. As CBP is sequestered into the aggregates during the pathogenesis of Huntington's disease, it is presumable that the circadian rhythm defects are caused by the loss of function of CBP. HD patients have disturbed day-night rhythm, fragmented and irregular sleep stages and increased wakefulness, that can lead to severe symptoms, which accelerate the progression of the disease. Therefore, understanding the defects of the underlying molecular mechanisms are essential.

To model Huntington's disease we expressed a toxic fragment of the human *Huntingtin* gene (*mHtt*) with expanded polyQ coding domain (Q120) in neuronal tissues of *Drosophila melanogaster*. In our model all the characteristic symptoms such as aggregate formation and neurodegeneration, reduced viability and lifespan, motor function abnormalities and circadian rhythm defects can be investigated.

Aims

In our research we used a *Drosophila* model of Huntington's disease (HD flies) to investigate such pathogenic processes of HD in which disturbed epigenetic regulation plays an important role.

- **As proper acetylation state of histone proteins has a major effect on the pathogenesis of HD our aim was to investigate the effects of epigenetic mark mimetic modifications of potential acetylation target positions by mutational analysis of the H3.3A histone variant (*H3.3A-mut*). To achieve this aim we performed the following experiments:**
  - Validation of nuclear transport and chromatin binding of mutant H3.3A histone variants.
  - Investigation of the effect of mutant H3.3A variants on the viability of wild type flies.
  - Investigation of the effect of mutant H3.3A variants on the viability, longevity, neurodegeneration, motor function and daily activity of HD flies.
  - Validation of the identified potential acetylation target positions by investigating the effect of loss of function of histone acetyltransferase and histone deacetylase enzymes.

- Transcriptome analysis of HD flies, and analysis of the transcriptional effects of mutations of the identified potential acetylation target positions.
- Investigation into the effects of mutations of the identified potential acetylation target positions on the total RNA/ DNA and polyA mRNA/ total RNA ratios.

- **Our other aim was to investigate the role of the CBP histone acetyltransferase in the regulation of circadian rhythm defects observed during the manifestation of Huntington's disease. To achieve this aim we performed the following experiments:**
  - Detailed analysis of the circadian rhythm defects of HD flies.
  - Investigation of the expression of circadian regulatory genes in HD flies.
  - Investigation of the effect of silencing *dCBP* in healthy flies.
  - Investigation of the effect of overexpressing *dCBP* in HD flies.

Methods

- Validation of transcript levels of neuronally expressed *mHtt* and *H3.3A-mut* by **RT-qPCR**.
- Validation of proper nuclear transport and chromatin binding of mutant H3.3A histones mimicking post-translational modifications (PTM) by **histone salt elution** followed by **western blot analysis**.
- Investigation of the effects of mutant H3.3A transgenes on the viability of wild type flies by **eclosion analysis**.
- Investigation of the effects of mutant H3.3A on the viability, longevity, neurodegeneration, motor function and daily activity of HD flies by **eclosion** and **survival analysis**, **pseudopupil assay**, **climbing assay** and **Trikinetics activity measurement**.
- Validation of the identified potential acetylation target positions: investigation of the effect of loss of function of Gcn5 histone acetyltransferase and Sirt1 histone deacetylase by **eclosion** and **survival analysis** and **pseudopupil assay**.
- Transcriptome analysis of HD flies, and investigating the transcriptional effects of mutations of the identified potential acetylation target positions by **RNA sequencing**.
- Investigation of the effects of mutations of the identified potential acetylation target positions on the ratios of total RNA/ DNA and

polyA mRNA/ total RNA by **RNA-DNA** and **polyA mRNA extraction**.

- Detailed analysis of the circadian rhythm defects of HD flies by **Trikinetics activity measurement**.
- Investigation of the expression of circadian regulatory genes in HD flies by **RT-qPCR**.
- Investigation of the effect of silencing *dCBP* in healthy flies and overexpressing *dCBP* in HD flies by **Trikinetics activity measurement** and **RT-qPCR**.

Major findings

- In these experiments we used PTM mimetic H3.3A transgenes with point mutations causing lysine to glutamine (K → Q, mimics acetylated lysine), lysine to arginine (K → R, mimics non-acetylated lysine), or lysine to methionine (K → M, mimics methylated lysine) substitutions. Mutant H3.3A histone variants mimicking PTMs are properly expressed, show nuclear localization and can be built into the chromatin.

- Except for H3.3A-K27M modification the mutations have no effect on the viability of wild type flies.

- The modifications of H3.3A-K9 and H3.3A-K27 positions have no remarkable influence on the pathogenesis of Huntington's disease.

- In the case of the acetylation mimicking H3.3A-K14Q modification improvement can be observed in viability, longevity and motor activity, the rate of neurodegeneration decreases, and hyperactivity caused by circadian rhythm defects is also moderated.

- The non-acetylated lysine mimicking H3.3A-K14R modification makes all the above-mentioned phenotypes of HD flies more severe.

- Heterozygous loss of function of the H3K14 specific Gcn5 histone acetyltransferase exacerbates HD phenotypes, while reduced level of Sirt1 histone deacetylase ameliorates them.

- Reduced level of *Gcn5* does not modify the positive effects of the acetylation mimicking H3.3-K14Q modification on HD phenotypes, while reduced level of *Sirt1* has a mild ameliorating effect.

- HD flies show remarkable transcriptional dysregulation. Approximately 90 % of the genes that show change in the expression level are downregulated, however, it is worthy of note that the expression of certain heat shock protein genes is upregulated.

- The gene expression pattern of HD flies is not influenced substantially by the PTM mimetic mutations of H3K14.

- Due to neurodegeneration the amount of DNA and total RNA decreases during aging in HD flies. However, the total RNA/ DNA ratio does not change, while the polyA mRNA/ total RNA ratio increases.

- In HD flies H3.3A-K14Q modification leads to increased total RNA/ DNA ratio is compared to the HD control, while H3.3A-K14R modification decreases this ratio. Interestingly the ratio of polyA mRNA to total RNA, reflecting the rate of transcription of coding genes is remarkably lower in both cases.

- HD flies show considerable hyperactivity while spend less time asleep and they have prolonged sleep-onset latency. Furthermore, fragmented sleep stages are also characteristic features of HD flies, as the number of sleep episodes is higher, however they are shorter in length.

- Remarkable changes can be observed in the gene expression pattern of *per*, *tim*, *vri* and *dClk* in HD flies, that presumably contribute to the circadian rhythm defects.

- Silencing of *dCBP* in healthy flies results in quite similar circadian rhythm defects that we observed in HD flies.

- Overexpression of *dCBP* in HD flies rescues both the sleep defects and the disrupted expression pattern of the circadian regulatory genes.

Summary

We investigated pathologic phenomena related to the reduced activity of histone acetyltransferase enzymes in a *Drosophila* model of Huntington's disease.

Histone acetyltransferase enzymes regulate transcription by maintaining the proper acetylation state of histone proteins, that is known to be dysregulated in Huntington's disease. By mutational analysis of several acetylation target positions of CBP and Gcn5 we identified H3K14 as a key acetylated target residue important in the pathogenesis, as mimicking the acetylated status of this position ameliorates, while mimicking the non-acetylated status worsens all investigated phenotypes. Our results suggest that maintaining the proper acetylation state of H3K14 might serve as potential therapeutic target in the treatment of the disease.

Furthermore, CBP also plays a role in the regulation of the circadian rhythm, which is also disturbed in Huntington's disease. Upon the loss of function of CBP quite similar circadian rhythm defects can be observed as in our *Drosophila* model of the disease, while its overexpression rescues these phenotypes in the HD model. However, the exact role of CBP in circadian rhythm regulation is yet to be cleared.

List of publications

MTMT ID: 10055600

Publications that form the basis of the Ph.D. thesis:

- **Faragó A**, Zsindely N, Bodai L. Mutant huntingtin disturbs circadian clock gene expression and sleep patterns in Drosophila. *Sci Rep*. 2019 May 9;9(1):7174. doi: 10.1038/s41598-019-43612-w. **IF: 4.149**

- Song W, Zsindely N, **Faragó A**, Marsh JL, Bodai L. Systematic genetic interaction studies identify histone demethylase Utx as potential target for ameliorating Huntington's disease. *Hum Mol Genet*. 2018 Feb 15;27(4):649-666. doi: 10.1093/hmg/ddx432. **IF: 4.544**

Further publications:

- **Faragó A**, Ürmösi A, Farkas A, Bodai L. The histone replacement gene His4r is involved in heat stress induced chromatin rearrangement. *Sci Rep*. 2021 Mar 1;11(1):4878. doi: 10.1038/s41598-021-84413-4. **IF: 3.998**

- Valkai I, Kénesi E, Domonkos I, Ayaydin F, Tarkowská D, Strnad M, **Faragó A**, Bodai L, Fehér A. The Arabidopsis RLCK VI_A2 Kinase Controls Seedling and Plant Growth in Parallel with

Gibberellin. *Int J Mol Sci.* 2020 Oct 1;21(19):E7266. doi: 10.3390/ijms21197266. **IF: 4.556**

- Spohn R, Daruka L, Lázár V, Martins A, Vidovics F, Grézal G, Méhi O, Kintses B, Számel M, Jangir PK, Csörgő B, Györkei Á, Bódi Z, **Faragó A**, Bodai L, Földesi I, Kata D, Maróti G, Pap B, Wirth R, Papp B, Pál C. Integrated evolutionary analysis reveals antimicrobial peptides with limited resistance. *Nat Commun*. 2019 Oct 4;10(1):4538. doi: 10.1038/s41467-019-12364-6. **IF: 12.121**

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Cumulative impact factor: 42.211