Histone methylation in Huntington's disease: are bivalent promoters the critical targets?

Huntington's disease (HD) is a currently incurable, late onset, progressive, ultimately fatal neurological disorder (Bates et al., 2015). We have recently published the results of comprehensive genetic interaction tests aimed at identification of histone methyltransferases and demethylases involved in HD pathogenesis in a Drosophila model of the disease (Song et al., 2018). The methylation state of histone proteins regulates the accessibility of chromatin structure by which it may influence transcriptional dysregulation observed in HD. We found that several factors affecting the methylation state of histone H3 lysine 4 (H3K4) and H3K27 residues influenced HD symptoms and identified the H3K27 specific demethylase, Utx, as a druggable target whose inhibition ameliorated neurodegeneration. These results in combination with previous findings suggest that bivalent chromatin regions, which are characterized by simultaneous presence of activating trimethylated-H3K4 (H3K4me3) and repressing H3K27me3 chromatin marks, might play a prominent role in HD pathogenesis. HD is caused by a dominant gain of function mutation of the huntingtin (HTT) gene, which encodes for the multifunctional Huntington (Htt) protein (Bates et al., 2015). The mutation is elongation of a polymorphic CAG trinucleotide repeat located in the 3′ untranslated region of HTT that encodes an expanded polyglutamine (polyQ) domain in the mutant Htt protein. Mutant Htt has widespread neuronal effects, however, medium spiny neurons (MSNs) of the striatum are particularly damaged by the disease. Mutant Htt is prone to aggregation and evokes a multifaceted pathology affecting the proteostasis network, intracellular transport and signaling, mitochondrial functions and transcriptional regulation (Bates et al., 2015). Due to its dominant monogenic nature HD lends itself to be studied in transgenic models of the disease (Song et al., 2018).

In the nuclei of mouse embryoid bodies wild-type Htt was found to localize to the subnucleolus, demonstrated by electron microscopy (Song et al., 2018). Drosophila is a well-suited organism to assess the contribution of genetic factors on HD as several critical targets of HD pathology are conserved in Drosophila and mouse. We have used a Drosophila model of HD disease (Song et al., 2018). Drosophila and mouse models of HD disease have shown that expression of dominant disease-causing mutant Htt protein causes dosage-dependent neuronal toxicity, as well as regional scale and neuronal type-specific gene expression changes (Dong et al., 2015). The results of this study imply that enzymes affecting protein methylation might play specific roles in neurodegenerative processes that cannot be simplified to generic effects on gene activation or repression. Analogously to our previous result that reduced H3K4me3 specific demethylase activity suppressed mutant Htt induced neurodegeneration (Vashishtha et al., 2013) we found that reduction of two H3K4 specific lysine demethylases, Kdm2 and Su(var)-3-3 (orthologs of human KDM2B and LSD1, respectively) also have similar effects. Surprisingly, however, reduction of two of the three H3K4 specific Drosophila methyltransferases, Trithorax and Trithorax-related (orthologs of MLL1/2 and MLL3/4, respectively), also ameliorated neurodegeneration (Song et al., 2018).

Previously we reported that the level of H3K4me3, a characteristic mark of active transcriptional start sites (TSS), was decreased on promoters of downregulated genes both in R6/2 mice and human samples, and the expression level of JARID1C, a H3K4me3 specific demethylase, was increased (Vashishtha et al., 2013). In R6/2 mice genes with reduced H3K4me3 expression had fission yolk gene ontology terms associated with neuronal functions and interestingly, more than half of down-regulated genes in the cortex of 12 weeks old mice were associated with a specific methylation pattern characterized by a broad distribution of H3K4me3 downstream of TSS. Partial knock-down of the JARID1C homolog little imaginal disks (lid), ameliorated mutant Htt induced phenotypes in Drosophila larvae, rescuing both neuronal loss and overt toxicity.

By comparing human HD and control prefrontal cortex samples by ChiP-Seq 720 differentially H3K4 trimethylated TSS proximal peaks were identified by Dong et al. (2015), most of which were lower in HD samples. Surprisingly, however, in spite of the overall positive correlation between H3K4me3 levels and gene expression in these samples, only 58 of those 720 genes that had differential enrichment of H3K4me3 at TSS proximal regions also showed differential gene expression levels, and only one third of the gene expression changes were in the expected regulatory direction. The above results combined suggest that H3K4me3 marks might be either improperly deposited or their regulatory influence on transcription might be debilitated or misinterpreted, and factors participating in H3K4 methylation might affect neurodegeneration by setting the methylation state of specific genes or gene groups rather than by universally influencing transcriptional output of genes.

In our test we also found that factors affecting the H3K27me3 epigenetic mark characteristic for suppressed regions influence HD pathogenesis (Song et al., 2018). Loss of three heterochromatin proteins (orthologs of H3K27me3 specific demethylase, UTX, Kdm2 and Su(var)-3-3) by genetic manipulation of the Drosophila model of the disease (Song et al., 2018), including homozygous mutations of the H3K27me3 specific demethylase, Utx, reduced both neurodegeneration and overt toxicity.

In vitro cell culture experiments also reported that expression of the H3K27me3 specific demethylase UTX significantly ameliorated neurodegeneration in R6/2 mice (Davidson et al., 2013). In our test we also found that factors affecting the H3K27me3 epigenetic mark characteristic for suppressed regions influence HD pathogenesis (Song et al., 2018). Loss of three heterochromatin proteins (orthologs of H3K27me3 specific demethylase, UTX, Kdm2 and Su(var)-3-3) by genetic manipulation of the Drosophila model of the disease (Song et al., 2018), including homozygous mutations of the H3K27me3 specific demethylase, Utx, reduced both neurodegeneration and overt toxicity.
ulators of MSN-specific functions, including neurotransmitter receptors, signaling proteins and MSN-specific transcription factors was reduced. In parallel with transcriptional dysregulation PRC2 deficient mice develop a progressive and fatal neurodegenerative phenotype with impaired motor functions and balance, decreased number of striatal MSNs and reduced total brain mass. The observed neurodegenerative changes provoked by loss of PR2C in MSNs were similar to alterations that are characteristic of HD with 20–30% of upregulated and 50% of downregulated genes in PRC2 deficient MSNs overlapping with genes up- and downregulated in brains of HD patients or mouse models of the disease. The majority of upregulated genes in PRC2 deficient MSNs were associated with a bivalent chromatin state displaying simultaneous presence of H3K4me3 and H3K27me3 epigenetic marks in wild type MSNs, suggesting that bivalent chromatin might have a pivotal role in neurodegeneration associated transcriptional dysregulation.

Interestingly, UTX, the H3K27me3 demethylase whose ortholog induced mutant Htt induced neurodegeneration in our analysis (Song et al., 2018) also has a prominent role in the regulation of bivalent promoters (Dhar et al., 2016). Utx was found to be recruited to and required for the activation of several retinoic acid inducible bivalent gene promoters in mouse embryonic stem cells (ESCs). Similar to UTX, H3K4me3 and H3K27me3 levels on bivalent promoters (Dhar et al., 2016). UTX and the H3K4 specific methhyltransferase, MLL2, are subunits of the ALR/MLL multiprotein complex that mechanistically links the removal of repressive and addition of positive chromatin marks (Issaeva et al., 2007).

Analysis of chromatin signatures in HD and control human cortical samples support the potential interplay of the H3K4me3 and H3K27me3 marks. In these samples around one third of H3K4me3 enriched regions were found to be located distally of known TSSs. These distal H3K4me3 peak regions are enriched for regulatory chromatin marks and partially overlap with known enhancers. In those distal H3K4me3 peak regions, which had elevated H3K4me3 levels in HD, binding sites of two PRC2 subunits, EZH2 and SUZ12, were found to be enriched (Dong et al., 2015). This suggests that debilitated PRC2 functions might contribute to increased H3K4 trimethylation at these regions in HD.

In conclusion, emerging data indicate that altered histone methylation patterns contribute to transcriptional dysregulation observed in HD. The involvement of both factors affecting activating H3K4me3 and ones affecting repressive H3K27me3 marks in HD pathology imply that perturbation of the dynamic interplay of H3K4 and H3K27 (de)methylation that fundamentally influences the activity of bivalent genes might contribute to pathogenesis. We hypothesize, that decreased level of repressive soluble Htt in HD might lead to reduced PRC2 activity and consequent decrease of H3K27me3 levels on bivalent promoters. In turn, improper release of bivalent promoters from repression might result in the dysregulation of cell-type-specific transcriptional programs that might contribute to degeneration (Figure 1). If this hypothesis proves to be true that will greatly improve our understanding of the transcriptional effects of HD by connecting epigenetic alterations that are currently considered separately. To achieve this, analysis of the effects of mutant Htt on bivalent chromatin with its associated epigenetic changes is imperative. Furthermore, pharmacological inhibition of UTX, that regulates bivalent promoters by H3K27 demethylation as a subunit of a H3K4 methyltransferase complex, might correct these alterations and holds promise as a therapeutic approach.

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