Background: The amino terminal region of Htt can be SUMO modified and this modification appears relevant to HD pathogenesis in vivo. Our goal is to establish the hierarchy of SUMO modification in the amino terminal region and identify key SUMO enzymes involved. Identification of the specific enzymes that play a role in SUMOylation of Htt may provide novel therapeutic targets for treatment of HD.
Methods/Techniques: To establish hierarchy, the three modifiable lysines (K6, K9, K15) present within Httex1 were mutated singly or in combination, purified, and evaluated using an in vitro SUMOylation assay, followed by mass spectrometry analysis. To identify candidate Htt-SUMO enzymes, His tagged proteins were purified under denaturing conditions and analyzed using a quantitative western blotting technique. To determine expression and distribution of the SUMO enzymes, mRNA levels in R6/2 cortex and striata were analyzed using qRT-PCR.
Results/Outcomes: Our results reveal site-specific modifications and establish a hierarchy of Lys 6 as the preferred SUMO modified lysine followed by Lys 9. SUMO enzymes evaluated for their ability to increase/decrease SUMO modification of Htt revealed that a single PIAS enhances and two SENPs reduce SUMOylation of Htt, suggesting these enzymes may be relevant for Htt SUMOylation. Preliminary knockdown data validates this possibility as do qRT-PCR results indicating that these SUMO modification enzymes are highly expressed in mouse brain. Experiments are ongoing to evaluate the functional significance of Htt SUMOylation in HD and extend analyses to longer forms of Htt.
Conclusions: Experimental results reveal selectivity for site-specific SUMO modification within the amino terminal region of Htt and specificity among SUMO modification enzymes. Together with differential expression patterns in brain, these SUMO enzymes are potential targets for treatment in HD.

A18 RECURRENT ALTERATION IN CHOLESTEROL BIOSYNTHESIS IN R6/2, YAC AND KIN MICE, HD RATS AND PRIMARY CELLS FROM HD MICE

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Background: Huntington disease (HD) cell lines, brains from HD mice and post-mortem cerebral tissues and primary fibroblasts from HD patients show a reduction in mRNA levels of key-cholesterogenic genes and in cholesterol biosynthesis activity. The underlying molecular mechanism entails hampered activity of SREBP transcription factor in HD cells.
Methods/Techniques: Isotopic dilution Mass Spectrometry, cellular, molecular and biochemical assays have been used to measure different parameters related to cholesterol biosynthesis and efflux in vivo and in vitro.
Results/Outcomes: We have measured the activity of the cholesterol pathway in brain samples from a number of transgenic mice and rats. Here we show that lathosterol levels are reduced in brain samples from YAC46, YAC72 and YAC128 mice in a CAG dependent manner. Reduced levels of lathosterol have also been found in the brain of HdhQ111 K-IN mice compared to WT HdhQ7/7 mice and in the brain of transgenic HD rats with respect to control samples. The same data has been obtained in brain from R6/2 mice. Other cholesterol precursors, cholesterol itself and the brain-specific catabolite 24-hydroxycholesterol (24OHC) decrease similarly in all HD samples tested which included R6/2, YAC and KIN mice and HD transgenic rats. Preliminary results also suggest that cholesterol biosynthesis is specifically reduced in primary astrocytes from YAC128 mice with respect to control cells. Notably, primary astrocytes from YAC18 mice, over-expressing normal huntingtin, show increased mRNA levels of key-cholesterol biosynthesis genes compared to wt and more if compared to mutant cells.
Conclusions: Overall these data highlight that cholesterol biosynthesis is reduced in the adult brain of different HD animal models and that glial cells may be the source of such a defect.
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A19 HSP70 AND HSP40 INTERPLAY ATTENUATES FORMATION OF SOLUBLE MUTANT HUNTINGTIN OLIGOMERS

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Background: A pathological hallmark of Huntington's disease (HD) is the aggregation of misfolded mutant huntingtin (mHtt) in inclusion bodies in the nucleus and cytosol of neurons. Molecular chaperones, especially members of the Hsp70 and Hsp40 families, co-localize to inclusion bodies and are neuroprotective in animal models of HD. Precisely how molecular chaperones suppress mHtt toxicity in vivo is unclear, but it may be due to direct effects of the chaperones on mHtt misfolding and aggregation.
Methods and Results: To analyze the effect of molecular chaperones on mHtt misfolding and aggregation, we used size-exclusion chromatography and atomic force microscopy (AFM). We show that mHtt fragments assemble into soluble oligomeric species with a broad distribution of sizes and morphologies. A subset of soluble oligomers reacts with the conformation-specific antibody A11, which previously had been shown to detect oligomers but not monomers or fibrillar aggregates formed by a polyglutamine peptide. This subset of oligomers had a globular structure as shown by AFM analysis. We found that Hsp70 and its co-factor Hsp40 co-fractionate with soluble mHtt oligomers and inhibit formation of A11-reactive oligomers in an ATP-dependent manner. Moreover, overexpression of Hsp70 and Hsp40 in a PC12 cell model suppressed formation of mHtt oligomers that react with A11.
Conclusion: These findings indicate that, in addition to recognizing mHtt monomers, Hsp70 and Hsp40 also interact with

