PIAS1 in Striatal SUMOylation Networks in Huntington's Disease

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Abstract

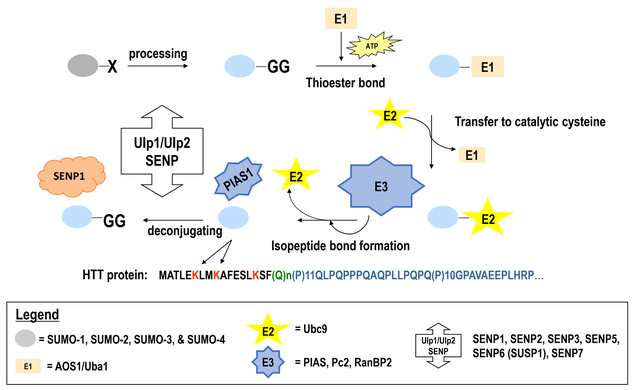
Huntington's disease (HD) is a devastating neurodegenerative disease characterized neuropathologically by structural atrophy of the striatum and cortex. This degeneration is caused by an expansion of a CAG-repeat motif in the huntingtin gene (*HTT*), resulting in synaptic dysfunction and neuronal death that overwhelmingly affects corticostriatal circuitry. SUMOylation, the attachment of small ubiquitin-like modifier (SUMO) proteins to target proteins, including HTT, is a prominent post-translational modification found to be dysregulated in HD. We identified a SUMO E3 ligase, PIAS1, that enhances SUMO modification of HTT. We propose that HTT, PIAS1, and SUMO coordinate regulation of proteostasis and synaptic function, so we developed a means for enriching SUMOylated proteins to investigate the global SUMO proteome in HD mouse and cell models. Our preliminary proteomic studies identified in mouse striata over 2000 proteins enriched from SUMO capture, several of which corresponded to synaptic, metabolic, structural, and chaperone proteins. This dataset represents SUMO-modified proteins, non-covalent interactions with SUMOylated protein complexes, and any other transient interactions with SUMO. Additionally, considering that both SUMO and PIAS1 are implicated in the DNA damage response, we investigated the interaction between PNKP, a DNA repair enzyme, SUMO, PIAS1, and HTT using our specialized SUMO capture technique. We found levels of di- and tri-SUMOylated species of PNKP were increased in HD iPSC-derived neurons compared to controls and upon PIAS1 knockdown, levels were normalized. Both unmodified and SUMO-modified species of PIAS1 accumulated in HD neurons more heavily than controls, supporting our previous data from PIAS1 KD studies in R6/2 mice. Although there was no difference in SUMO-modified full-length HTT, HD neurons displayed increased levels of SUMOylated proteins, with PIAS1 KD reversing this increase.

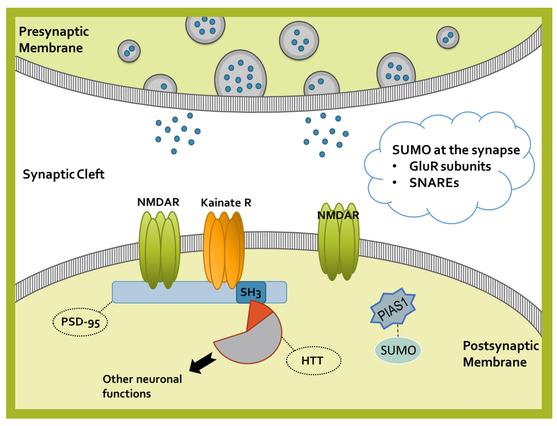
Background & Rationale

SUMOylation plays an important role at the HD synapse

Hypothesis: PIAS1 is a link between protein homeostasis and synaptic function in HD by modulating key synaptic proteins

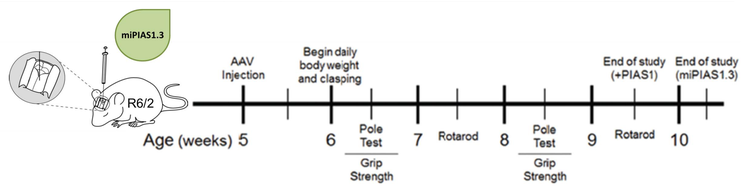
SUMOylation Enzymatic Cascade





Examining the therapeutic potential and impact of targeting PIAS1 using the R6/2 HD mouse model

**Investigate disease-related networks following modulation of PIAS1 in R6/2 HD-modeled mice*



PIAS1 knockdown in HD striatum restores disease-associated pathways

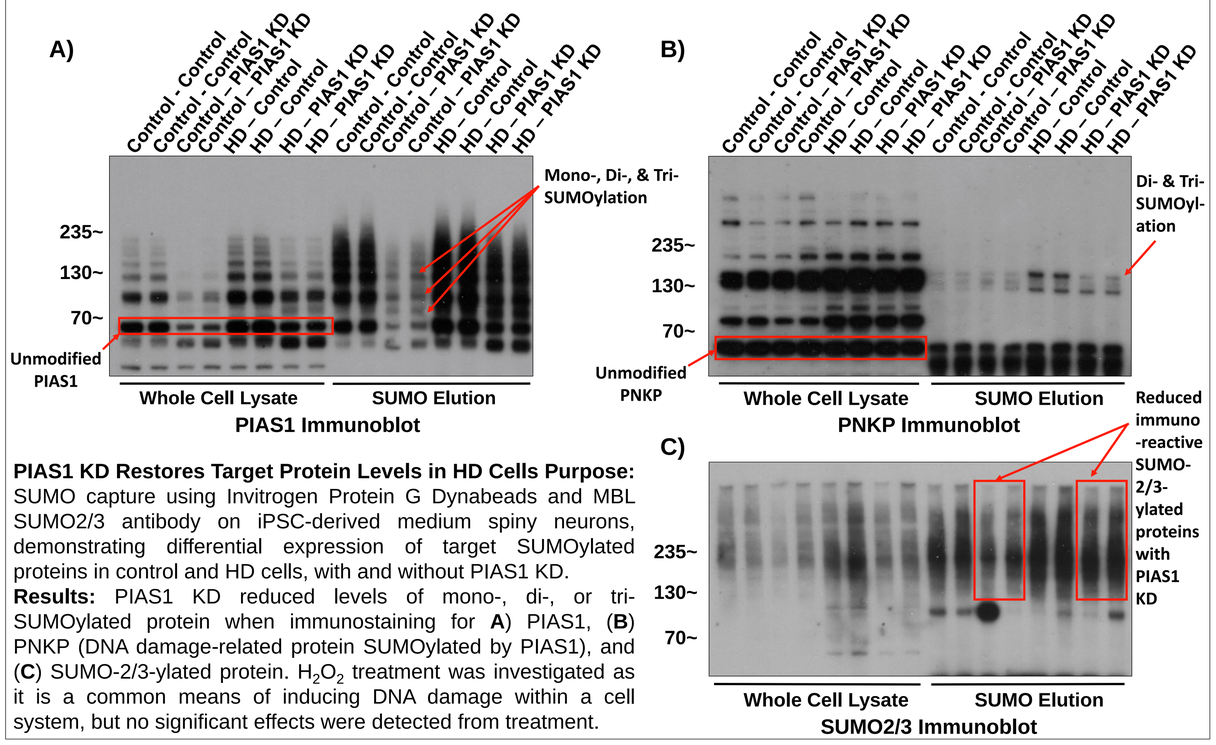
Purpose: Examine miPIAS1.3 treatment on striatal pathology (mHTT accumulation and aggregation) and synaptic markers.
Results: Treatment with miRNA-mediated PIAS1 knockdown (KD) at 5 weeks of age significantly reduces the accumulation of:

- insoluble high molecular weight accumulated mHTT
- Proteins post-translationally modified by SUMO and ubiquitin
- intranuclear inclusion bodies

Additionally, PIAS1 KD increases levels of synaptophysin in HD mice compared to miSAFE treated animals. PIAS1 may represent a multifunctional protein target in regulating several networks previously shown to be dysfunctional in HD at the cell body and synapse.

Ochaba, *et al.* Neuron (2016).

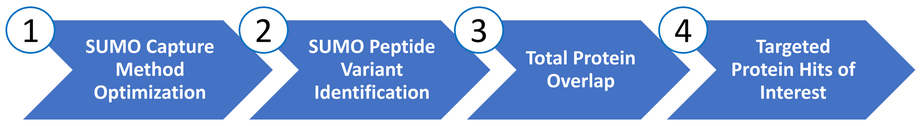
SUMO capture of selected protein targets following PIAS1 KD in HD iPSC-derived medium spiny neurons

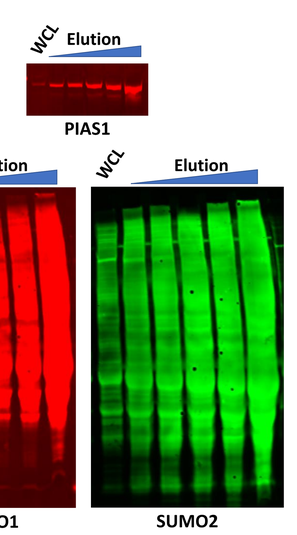


PIAS1 KD Restores Target Protein Levels in HD Cells Purpose: SUMO capture using Invitrogen Protein G Dynabeads and MBL SUMO2/3 antibody on iPSC-derived medium spiny neurons, demonstrating differential expression of target SUMOylated proteins in control and HD cells, with and without PIAS1 KD.
Results: PIAS1 KD reduced levels of mono-, di-, or tri-SUMOylated protein when immunostaining for **A**) PIAS1, (**B**) PNKP (DNA damage-related protein SUMOylated by PIAS1), and (**C**) SUMO-2/3-ylated protein. H_2O_2 treatment was investigated as it is a common means of inducing DNA damage within a cell system, but no significant effects were detected from treatment.

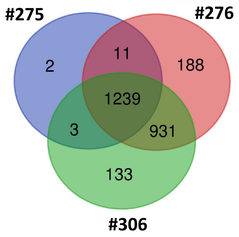
Preliminary Results

Method optimization for enrichment of SUMOylated proteins from HD mouse striata



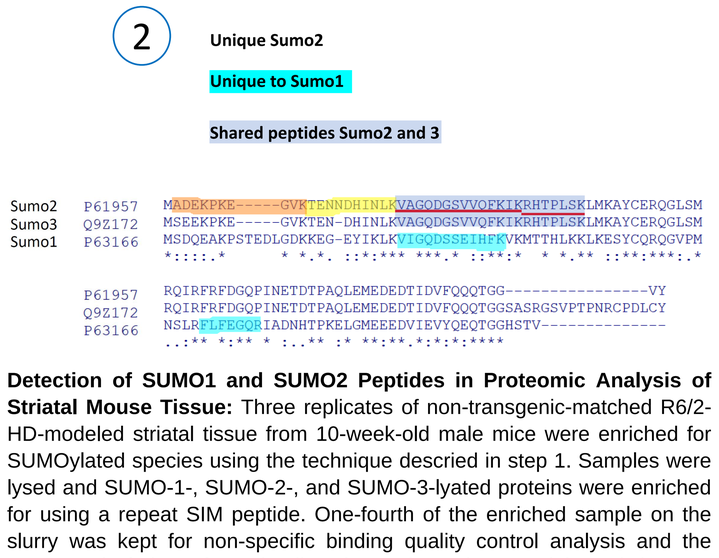


An Antibody-less Approach to SUMO Capture: Immunoblots demonstrating the capacity for capturing SUMOylated protein from wild-type striatal mouse tissue. The Enzo SUMO Qapture-T technique utilizes a peptide with repeating SUMO interacting motifs (SIMS) to capture SUMO-1, -2, and -3-ylated species. SUMO capture of PIAS1 shows a non-covalent interaction with SUMO

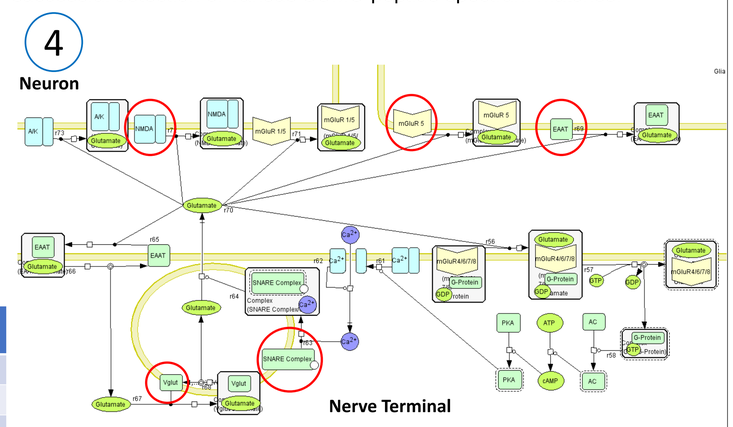


MS Sample Name	Total Proteins	Total Peptides	Total Spectra
#275	1255	4935	6400
#276	2369	14422	17499
#306	2306	13190	15659
Total Unique	2507	16923	31515

Three-way Protein Identification Summary between Samples: SUMOylated proteins identified by mass spectrometry demonstrates a large overlap between replicates, with over 2000 identified in total.



Detection of SUMO1 and SUMO2 Peptides in Proteomic Analysis of Striatal Mouse Tissue: Three replicates of non-transgenic-matched R6/2-HD-modeled striatal tissue from 10-week-old male mice were enriched for SUMOylated species using the technique descried in step 1. Samples were lysed and SUMO-1-, SUMO-2-, and SUMO-3-lyated proteins were enriched for using a repeat SIM peptide. One-fourth of the enriched sample on the slurry was kept for non-specific binding quality control analysis and the proper calculation of tryptic digestion ratios. The remaining 3/4ths was digested directly on the slurry matrix using a starting amount of 1.5 ug trypsin. Mass spectral analyses of the samples were acquired using the Lumos orbitrap instrument at a 120 minute gradient. Step 2 indicates the successful detection of various SUMO peptide spectrum matches.



Identification of SUMOylated Protein Targets of Interest within Select Gene Ontology Pathways: Several proteins were identified within the Metabotropic Glutamate Receptor Group III Pathway (P00039) from the Panther Classification System, including several NMDA receptor subunits, metabotropic glutamate receptors, vesicular glutamate transporters, and the SNARE assembly complex.

Conclusions

- We have successfully devised a workflow process for enriching SUMOylated proteins from HD-modeled mouse brain tissue and HD-modeled induced pluripotent stem cells (iPSCs).
- This method has demonstrated feasibility and reproducibility for identification of SUMOylated proteins.
- Differences identified in SUMOylation networks by proteomic analysis in HD and PIAS1 KD mice will further elucidate the role of SUMOylation at the synapse in relation to HTT and cellular proteostasis in HD.
- These identifications may open the door towards informed mechanistic targets for therapeutic intervention in HD, including the development of a small molecule PIAS1 inhibitor and a path to analyze the SUMO-ome in neurodegeneration..
- Studies for identification of a PIAS1 inhibitor are concurrently being carried out using a thermal shift assay to determine stabilization of native protein structure upon small molecule ligand binding.

Support

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