DYSREGULATION OF POLY ADP RIBOSE SIGNALING IN HD and its potential as a therapeutic target

Huntington's Disease Society of America
Berman/Topper HD Career Development Fellowship

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HUNTINGTIN BINDS POLY ADP RIBOSE

BACKGROUND

- DNA repair pathways affect HD age at onset (GeM-HD Consortium, 2015)
- Huntingtin acts as a scaffold for DNA repair proteins (Maiuri et al, 2017)
- Poly ADP ribose (PAR) is a post-translational modification generated in response to DNA damage

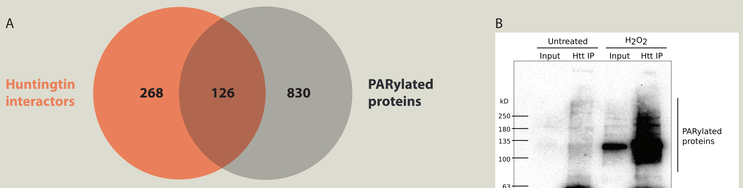
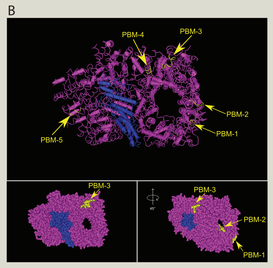


Figure 1: Huntingtin interacts with PARylated proteins
A. Degree of overlap between huntingtin-interacting proteins purified from *STHdh* cells and a compiled list of PARylated proteins from three independently generated databases (Gagne et al, 2008; Zhang et al, 2013; Jungmichel et al, 2013).
B. RPE1 cells were treated with 400 uM H_2O_2 for 10 min and proteins crosslinked with 1% PFA. Huntingtin was immunoprecipitated with EPR5526 and associated proteins separated by SDS-PAGE and immunoblotted with the indicated antibodies (MABE1016: PAR detection reagent).

A

UNIPROT	PROTEIN	SITES	CONSENSUS	CITATION
			b x x h b b h h	
P78527	DNA-PK	2732-2754	F M R D Q E K L S L M Y A R K G V A E Q K R E	Pleschke, 2000
P55957	BID	143-166	K E K T M L V L A L L L A K K V A S H T P S L	Gagne, 2008
O43684	BUB3	144-166	S V S G D R L I V G T A G R R V L V W D L R N	Gagne, 2008
O76075	CAD	143-165	E G L E S R F Q S K S G Y L R Y S C E S R I R	Pleschke, 2000
P10155	SS-A/Ro	137-158	E S M K C G M W G R . A L R K A I A D W Y N E	Gagne, 2003
Q00653	NFkB p100	169-191	E Q R E L E Q E A K . E L K K V M D L S I V R	Pleschke, 2000
P42858	HTT PBM-1	165-186	L P R L Q L E L Y K . E I K K N G A P R S L R	
	HTT PBM-2	1522-1543	K I I Q L C D G I M A S G R K A V T H A I P A	
	HTT PBM-3	1782-1804	L M C L I H I F K S G M F R R I T A A A T R L	
	HTT PBM-4	1884-1906	L A A K L G M C N R E I V R R G A L I L F C D	
	HTT PBM-5	2535-2556	N K P L K A L D T R . F G R K L S I I R G I V	

B



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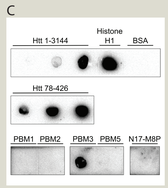
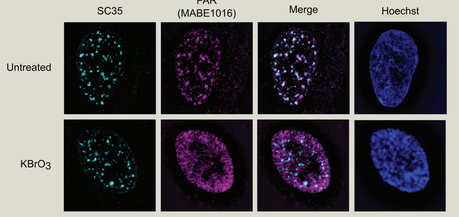


Figure 2: Huntingtin PAR-binding motifs
A. Huntingtin sequence analysis revealed five motifs matching the PAR-binding concensus.
B. PBM-1, 2, and 3 map to the surface of the huntingtin structure (Guo et al, 2018), while PBM-4 is not exposed. PBM-5 is exposed on the C-terminal domain.
C. Dot blot PAR overlay assay with full length purified huntingtin (Harding et al, 2019) (top), huntingtin fragment 78-426 (middle), and PBM peptides (bottom). Purified proteins were dotted onto nitrocellulose then overlaid with 0.2 uM PAR (Trevigen). After washing, anti-PAR western was performed with PAR detection reagent MABE1016.

A



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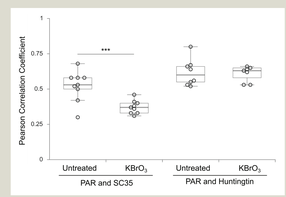

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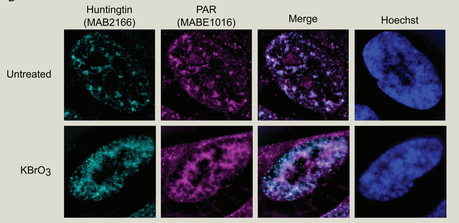


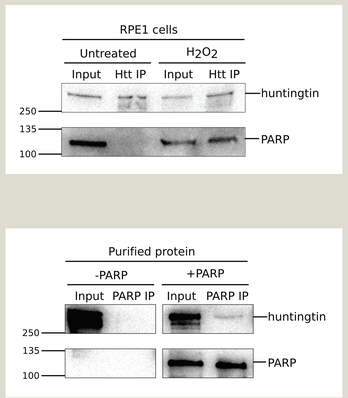
Figure 3: Huntingtin co-localizes with PAR
RPE1 cells were treated with 10 uM PARG inhibitor for 15 min followed by 100 mM $KBrO_3$ for 30 min. Soluble proteins were extracted with 0.2% Triton X-100 for 2 min on ice followed by fixation and staining with the indicated antibodies. Cells were imaged by super-resolution microscopy (SR-SIM).

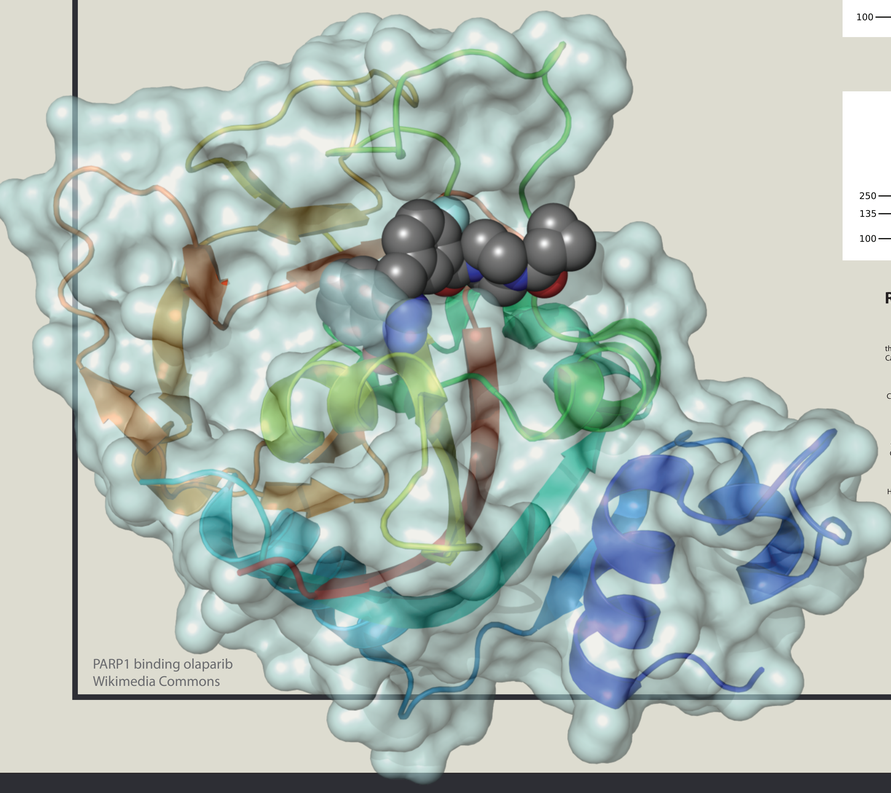
A. PAR co-localizes with SC35-positive nuclear speckles.

B. Huntingtin co-localizes with PAR at speckles and redistributes to sites of PAR production upon oxidative stress.
C. Co-localization of nuclear signals measured by Pearson correlation. n=1, 10 cells per condition.

HUNTINGTIN BINDS PARP

Figure 4: Huntingtin interacts with Poly ADP ribose polymerase
A. RPE1 cells were treated with 400 uM H_2O_2 for 10 min and proteins crosslinked with 1% PFA. Huntingtin was immunoprecipitated with EPR5526 (Abcam) and associated proteins separated by SDS-PAGE and immunoblotted with anti-PARP (BD Biosciences) and anti-huntingtin (MAB2166, Millipore).
B. Purified huntingtin (Harding et al, 2019) and PARP (Trevigen) were incubated in the presence of activated DNA and NAD+. PARP was immunoprecipitated with anti-PARP antibody (BD Biosciences). Reactions were separated by SDS-PAGE and immunoblotted as in A.





PARP1 binding olaparib
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ELEVATED PAR IN HD

BACKGROUND

- Levels of DNA damage are elevated in HD cells and tissues (Maiuri et al, 2017; Askeland et al, 2018; Castaldo et al, 2019)
- Unrepaired DNA damage leads to prolonged activation of PARPs and overproduction of PAR
- Unrelenting PAR production causes ATP depletion, mitochondrial failure and energy crisis
- PAR acts as a mediator of cell death through parthanatos

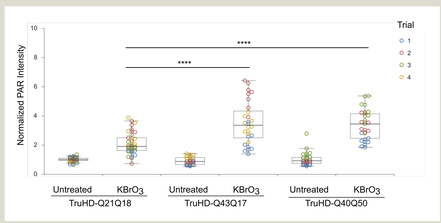

Figure 5: Elevated PAR levels in HD patient fibroblasts
TruHD immortalized HD patient and control fibroblasts (Hung et al, 2018) were pre-treated with 10 μM PARG inhibitor for 15 min followed by 100 mM $KBrO_3$ for 30 min. Soluble proteins were extracted with 0.2% Triton X-100 for 2 min on ice, followed by fixation and staining with MABE1016 PAR detection reagent. Nuclei were identified as primary objects in CellProfiler (Carpenter et al, 2006) using Hoechst staining, then pixel intensity of the PAR staining within nuclei was calculated and the mean intensity recorded for each image. Ten images per well were captured, representing 750-1000 cells per experiment. The experiment was repeated 3 times for TruHD-Q43Q17 and TruHD-Q40Q50 cells, and four times for TruHD-Q21Q18 cells. P-values were calculated using Tukey's test (****p<0.0001).

IS PARP INHIBITION A VIABLE THERAPEUTIC STRATEGY?

MORE PRECLINICAL DATA NEEDED

- PARP inhibition is beneficial in HD mouse model (Cardinale et al, 2015; Paldino et al, 2017)
- High PAR levels in HD patient fibroblasts (Fig 5)
- Are PAR levels high in CSF from HD patients?
- Does PARP inhibition rescue phenotypes in clinically relevant models?

REPURPOSING PARP INHIBITOR DRUGS

- Veliparib, niraparib cross the blood brain barrier
- Risk of genotoxicity, however PARP inhibitors are generally protective in postmitotic cells (Berger et al, 2018)

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