**Project:** PAR dysregulation in HD

**Experiment:** Phospho-N17-huntingtin immunofluorescence in PARG inhibitor-treated cells

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**Experiment 1**

**Seeding**

*2020-12-19*

Seeded RPE1s (p10) in drilled glass-bottom 6-well plate: 500 uL/10 mL from a 95% confluent 10-cm plate per well.

*2020-12-20*

Treated cells with either 1 uM talazoparib or 10 uM PARG inhibitor (PDD00017273 from AdipoGen Lifesciences) for approximately 1 h. As this was originally part of a micro-irradiation assay, treatment was done in growth media with NucBlue for the first 20 minutes followed by inhibitor only in HBSS.

**Fixation and staining**

* Fixed in cold methanol 10 min at -20
* Blocked in 10% FBS/PBS for 15 min at room temp
* Incubated with anti-P-N17 (1:500 dilution in blocking buffer 45 min at room temp)
* Washed twice with PBS
* Incubated with donkey anti-rabbit-594 (1:500 20 min at room temp)
* Washed twice with PBS
* Incubated with mouse anti-PARP1 (BD Biosciences, 1:500 1h at room temp)
* Washed twice with PBS
* Incubated with donkey anti-mouse-488 (1:500 20 min at room temp)
* Washed twice with PBS
* Incubated with 0.2 ug/mL Hoechst for 5 min
* Washed once with PBS and imaged in PBS on the Nikon A1 confocal microscope (60X objective)

**Results**

In PARG inhibitor-treated cells, mitotic cells had very striking staining pattern in which the huntingtin seemed to “coat” the condensed chromosomes. Staining in PARP inhibitor-treated cells resembled what is usually seen in untreated cells [1].

A picture containing graphical user interface

Description automatically generated

**Experiment 2**

*2020-12-28*

Seeded RPE1s in 8-well Ibidi dish for rather dense confluence to increase chances of finding mitotic cells:

* WT: brought 0.75 mL/10 mL from 95% 10-cm plate (p13) to 10 mL, plated 300 uL per well x 4 wells
* PARP1/PARP2 KO: brought 1.5 mL/10 mL from 95% 10-cm plate (p8) to 10 mL, plated 300 uL per well x 4 wells

*2020-12-29*

Next day, cells were not overly dense (~90%), but there were a few mitotic cells in the middle of each well (detected as rounded, healthy cells).

**Treatment**

* Added 10 uM PARG inhibitor in fresh growth media to each well for 20 min
* Replaced with 10 uM PARG inhibitor in fresh growth media containing NucBlue (one drop into 2 mL media) for 20 min
* Stress conditions:
  + No stress: HBSS + PARG inhibitor approx 20 min
  + KBrO3: PBS/Ca2+/Mg2+ containing 100 mM KBrO3 and PARG inhibitor approx 20 min
  + H2O2: PBS/Ca2+/Mg2+ containing 400 uM H2O2 and PARG inhibitor approx 20 min
  + UV: HBSS + PARG inhibitor 10 min followed by irradiation of 10 ROIs at 4 points (2 points in WT, 2 points in PKOs), which took approx 10 min

**Fixation and staining**

* Fixed in cold methanol 10 min at -20
* Blocked in 10% FBS/PBS for 15 min at room temp
* Incubated with anti-P-N17 (1:1000 dilution in blocking buffer) overnight

*2020-12-30*

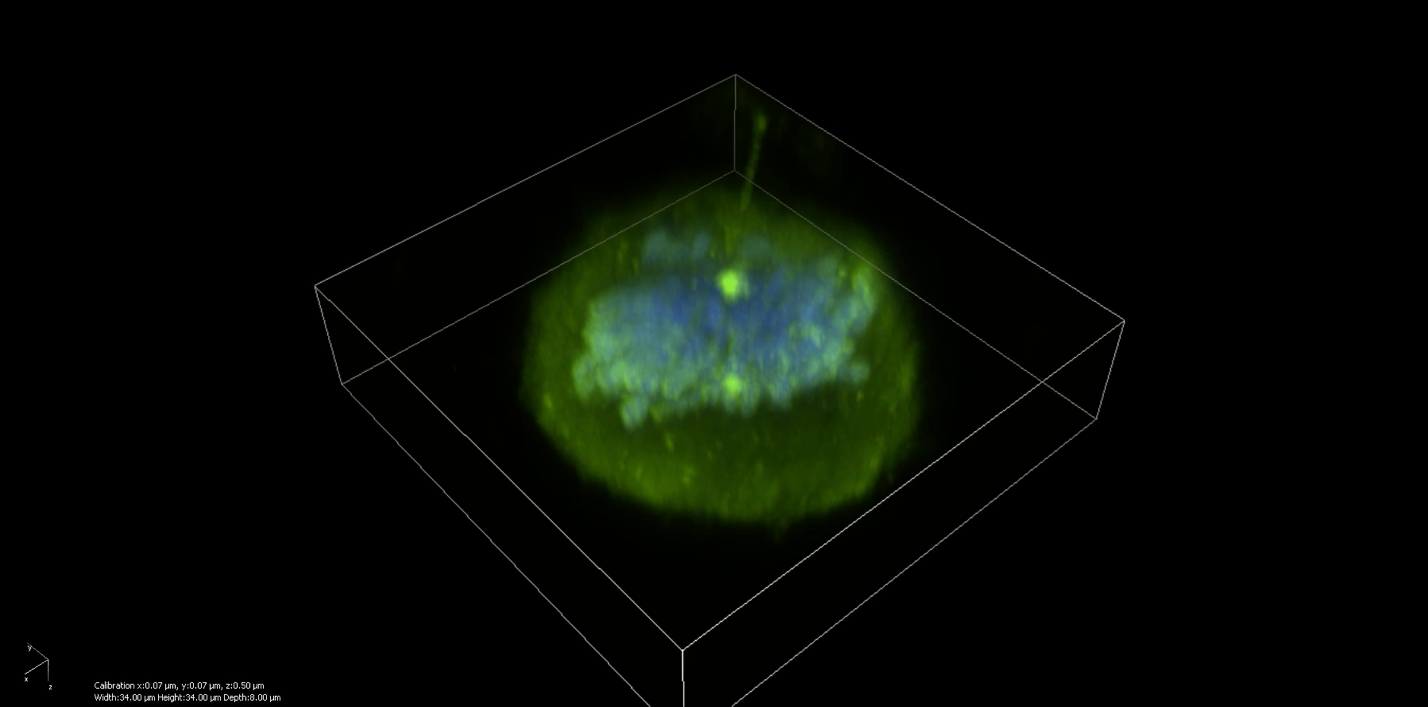
* Washed twice with PBS
* Incubated with goat anti-rabbit-488 (1:1000 dilution in blocking buffer) for 15 min at room temp
* Washed twice with PBS
* Incubated with 0.2 ug/mL Hoechst for 5 min
* Washed once with PBS and imaged in PBS on the Nikon A1 confocal microscope (60X objective)

**Results**

No mitotic cells could be found in any of the stress conditions, but found 2 mitotic cells in the no stress condition, which displayed the huntingtin-coating-chromosomes pattern:

A picture containing monitor, indoor, screen, dark

Description automatically generated



Double click to start movie: 3D reconstruction of Z-stack

**Conclusions**

* Huntingtin localizes to condensed chromosomes upon PARG inhibition
* Evidence that huntingtin binds PAR in vivo
* Can act as a readout for the PAR binding motif mutant, once CRISPR edited
* To do: try co-staining with PAR
* Using BenchSci, found these two images of IF against PAR in mitotic cells. Looks a lot like huntingtin in PARG inhibitor-treated cells:

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5114291/figure/fig03/>:

A green light on a black surface

Description automatically generated with low confidence

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC319445/figure/F3/>:

A picture containing text, night sky

Description automatically generated

**References**

1. Atwal RS, Desmond CR, Caron N, Maiuri T, Xia J, Sipione S, et al. Kinase inhibitors modulate huntingtin cell localization and toxicity. Nat Chem Biol. 2011;7: 453–460.