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C9orf72 poly GA RAN-translated protein plays a key role in amyotrophic lateral sclerosis via aggregation and toxicity

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Since this article was originally published, the authors discovered errors in Figures 3H and 6A. In Figure 3H the last lane GAPDH was excluded, while in Figure 6A, the western-blot shows 6 lanes for PARP and TDP-43 but 7 lanes have been included for GAPDH. The correct figures are included here.
Figure 3. Poly GA is the most abundant DPR in human cortex and is toxic to cultured cells. (A) The total numbers of DPR-positive cells were counted in the C9orf72-positive human frontal cortex (n=5). (a-e) Rabbit anti-DPR antibodies were used for the immunostaining for GA, GP, GR, PA, and PR (Green). DAPI (blue) was used for nuclear counterstaining, and (f) 50 cells were counted from each section (n=5). Scale bar 1 mm. (B) Schematic diagram of scrambled synthetic DPR, which incorporated start codon ATG. Artificially synthesized DPR DNAs were cloned into an EGFP expression vector, which generates N-terminal DPR-EGFP fusion proteins. Expression of DPR-EGFPs was tested in HEK-293 cells. Transcription of mRNA levels of transfected plasmids was assessed by semi-quantitative RT-PCR. (C) Western blot analysis of total cell lysates showed that poly GA forms a high molecular weight species. (D) Filter trap assay revealed that GA-EGFP, GR-EGFP and PR-EGFP are insoluble. An equal amount of lysates was loaded on nitrocellulose membranes. Membranes were stained with an anti-GFP antibody. (E) Expression of synthetic 125 repeat DPRs in HEK-293 cells results in the formation of cytoplasmic (GA-EGFP) or nuclear inclusions (GR-EGFP, PR-EGFP). In contrast, no inclusions were detected in GP-EGFP and PA-EGFP expressing cells. Scale bar 1 mm. (F) Western blot analysis of total cell lysates from DPR-expressing cells for anti-PARP (top) and anti-TDP-43 (middle) revealed that GA-EGFP produced PARP cleavage, a marker of cell death and a 37 kDa TDP-43 cleavage product. GAPDH was used to normalize the protein loading. (G) Quantitative analysis of PARP western blotting showed that cells bearing inclusions of GA have significantly increased cleaved PARP (***P<0.0001). (H) Dose-response of GA-EGFP (50-10000 ng/well) was assessed for PARP (top) and TDP-43 (middle) cleavage. The high molecular weight of GA-EGFP (bottom) was increased following the dose of GAEGFP plasmids.
Figure 6. Poly PA inhibit PARP cleavage and aggregation of poly GA. (A) Dual transfection of GA-EGFP with PA-EGFP decreases PARP cleavage. GA-EGFP (250 ng) with GP-EGFP, GR-EGFP, PA-EGFP and PR-EGFP (250 ng) were transfected in HEK-293 cells, and total cell lysates were used for PARP and TDP-43 (middle) analysis by western blotting. (B) Quantitative analysis of cleaved PARP showing that cells bearing inclusions of GA/PAPA have significantly decreased levels of cleaved PARP (***P < 0.0001). (C) Dual (DPR-EGFPs Þ GA-EGFP) transfected HEK-293 cells were stained with anti-GA antibodies (red). Blue represents nuclear counterstaining of DAPI. Scale bar 1/4 10 μm. (D) Quantitative analysis of GA-EGFP positive cells showing that cells are bearing inclusion of GA, PA have significantly decreased GA-EGFP aggregates (***P < 0.0001).