# Transgenic expression of mutant huntingtin sensitizes human neuroblastoma cells to oxidative stress: involvement of Autophagy

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***Objectives:***

*Our overall research objective is to expand the current knowledge and unravel key pathways underlying autophagy, in Huntington's Disease (HD). De facto, the pro-survival or pro-death contribution of autophagy in neurodegenerative diseases is controversial. HD, an autosomal dominant movement disorder, is caused by the expansion (>35 repeats) of CAG trinucleotide repeats in the huntingtin (htt) gene, which encodes an expanded polyglutamine (poly(Q)) tract in the N-terminus of the Htt protein. The toxicity of the mutant htt protein is believed to result from the intraneuronal aggregates of the N-terminal fragments, the typical pathological hallmark of HD (Di Figlia et al., 1997) . Furthermore, wild-type Htt, a large protein widely expressed in neurons, has many functions including anti-apoptotic, vesicles trafficking/endocytosis and scaffold protein for selective macroautophagy. The mutant polyQ Htt is aggregate-prone and may affect the endocyitc traffic and autophagy (Rubinzstein, 2002). Autophagy is a catabolic process that plays a crucial role in cell homeostasis through the lysosomal digestion of dysfunctional organelles and protein aggregates. Cathepsin D (CD) is an aspartic lysosomal protease mainly involved in the degradation of misfolded proteins (among which Htt) targeted by the autophagy system (Qin et al., Hum Mol Gen 2009; Liang et al., Mol Neurobiol 2011). Oxidative stress is a well known cause of protein aggregation (Fulda et al., 2010). Dopamine (DA) is reported to induce oxidative stress in neurons and to trigger the endosomal-lysosomal system (Cagnin et al., Brain Res. 2012). Interestingly, alteration in DA balance in the striatum leads to pathological conditions, such as abnormal movements and cognitive deficits in HD (Chen et al., 2013).*

## MATERIALS and METHODS

SH-SY5Y dopaminergic human neuroblastoma cells were used as a model for the endogenous Htt and the transgenic expression of normal and mutant PolyQ Htt.

Cells were transfected with different HD constructs in pcDNA3:

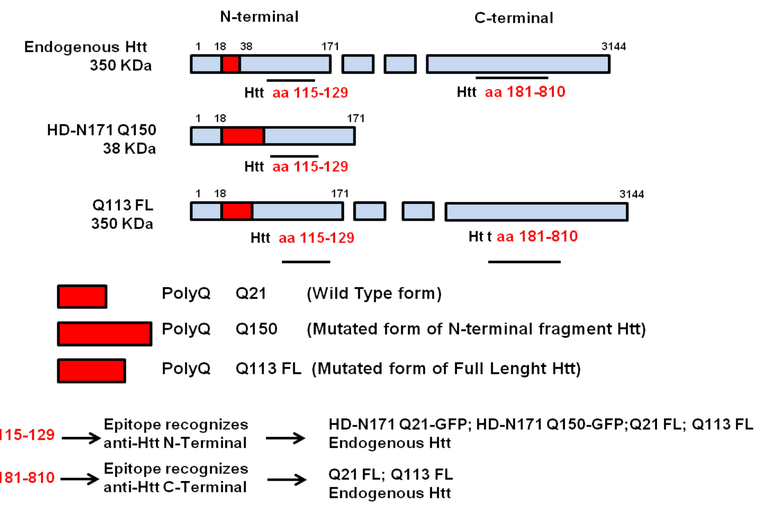
a)HD-N171Q21-GFP and HD-N171Q150-GFP, which encode the amino-terminal 171 amino acids fragment of human huntingtin protein, with 21 and 150 glutamines (9).

b)Q21 FL and Q113 FL, which encode the full length human huntingtin protein, with 21 and 113 glutamines, respectively.

Inhibitors of the autophagy-lysosomal proteolytic pathway: 30 μM of Chloroquine (ClQ; blocks the fusion between autophagosome and lysosome and increases the lysosomal pH); 100 μM Pepstatin (PstA; a specific inhibitor of Cathepsin D.

Cells were also incubated with 20 μM z-VAD-fmk (pan-caspase inhibitor). Cells were incubated with 100 μM Dopamine (DA), a catecholamine neurotransmitter.

The autophagy system and the fate of endogenous and transgenic Htt, both fragments and full length protein, were studied by Western blotting and Immunofluorescence analysis. Cell death was assessed by counting Trypan blue-stained cells and by Propidium Iodide (PI) staining in not-fixed cells (necrotic cells).




## RESULTS

### Endogenous Htt is degraded by Autophagy

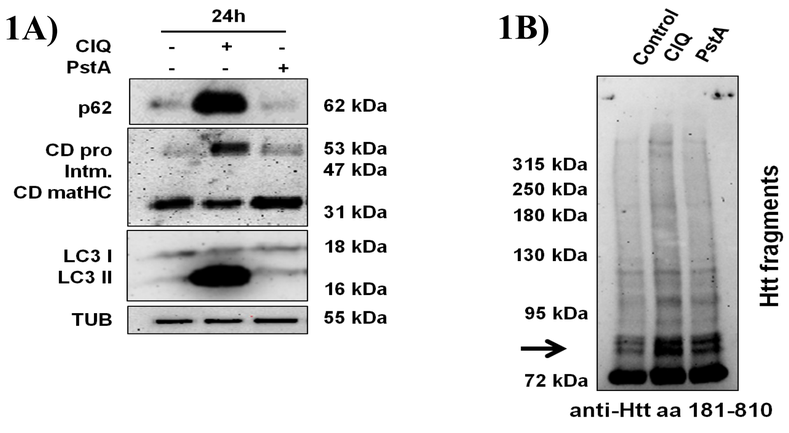
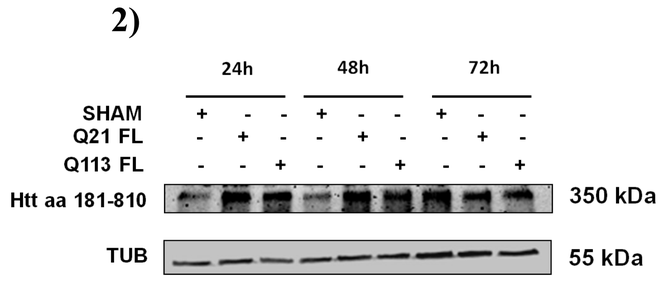



**Fig. 1**: Incubation with ClQ and PstA inhibits the autophagy-lysosome degradation (**A**) and leads to the accumulation of 72-100 kDa Htt fragments (**B**).

### Expression of Q113 FL affects cell growth




DOUBLING TIME

| | SHAM | Q21 FL | Q113 FL |
|---|---|---|---|
| T d1 | 31,60 ± 2,46 | 35,44 ± 3,25 | 51,74 ± 6,74 |
| T d2 | 77,28 ± 5,09 | 78,33 ± 10,87 | 106,60 ± 14,89 |

**Fig. 2**: In Q113 FL expressing neuroblastoma cells, the doubling time is two-folds longer than in SHAM and Q21 FL expressing cells, indicating that the expression of mutant Htt protein interferes with cellular duplication.

### Expression of Q113 FL sensitizes to DOPAMINE toxicitv

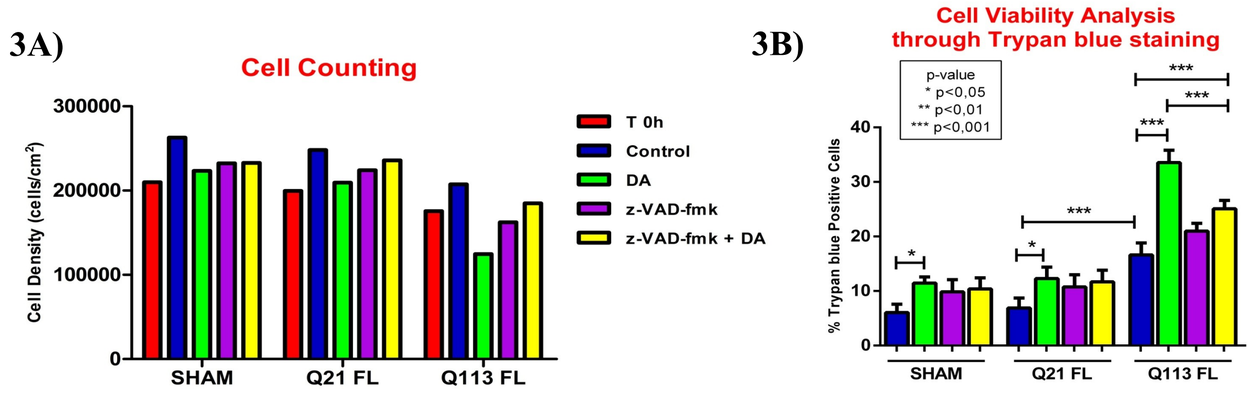



Fig. 3A: Cell growth in Q113 FL expressing cells decreases compared to SHAM and Q21 FL expressing cells.
Fig. 3B: Cell death in Q113 FL expressing cells, DA toxicity is exacerbated three-times more than SHAM and Q21 FL cells (Q21FL + DA vs Q21FL Control = 40% mortality; Q113 FL + DA vs Q113 FL Control = 135% mortality).
Co-incubation with z-VAD-fmk partially rescues cell viability in Q113 FL transfected cells exposed to DA.

### DOPAMINE induces necrosis in HD-N171 Q150 expressing neuroblastoma cells

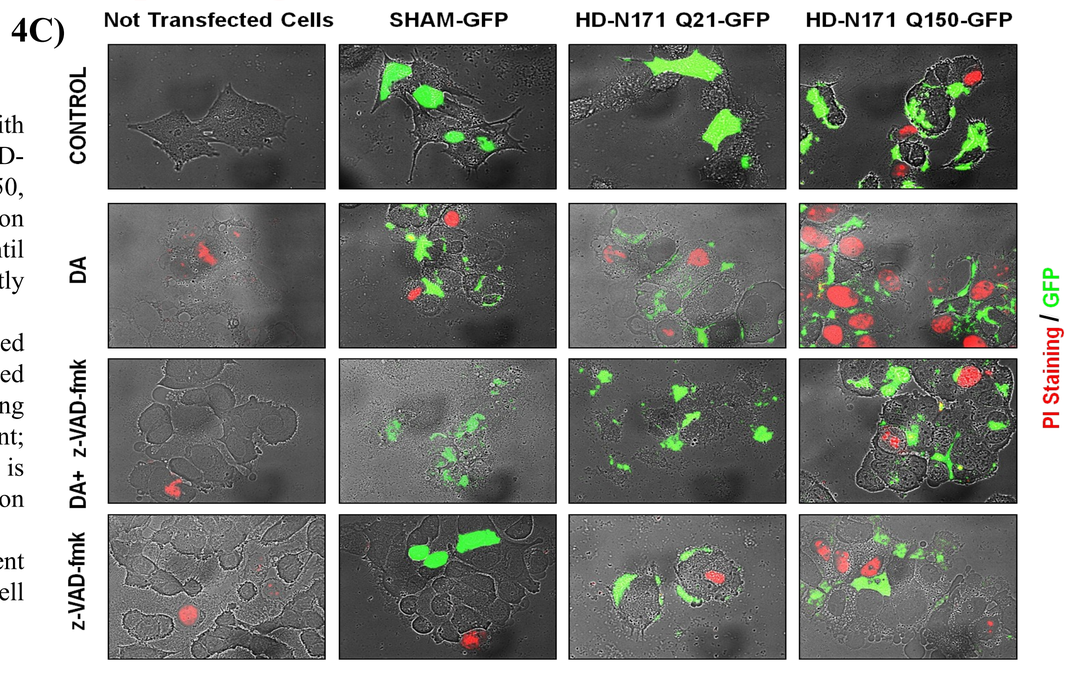
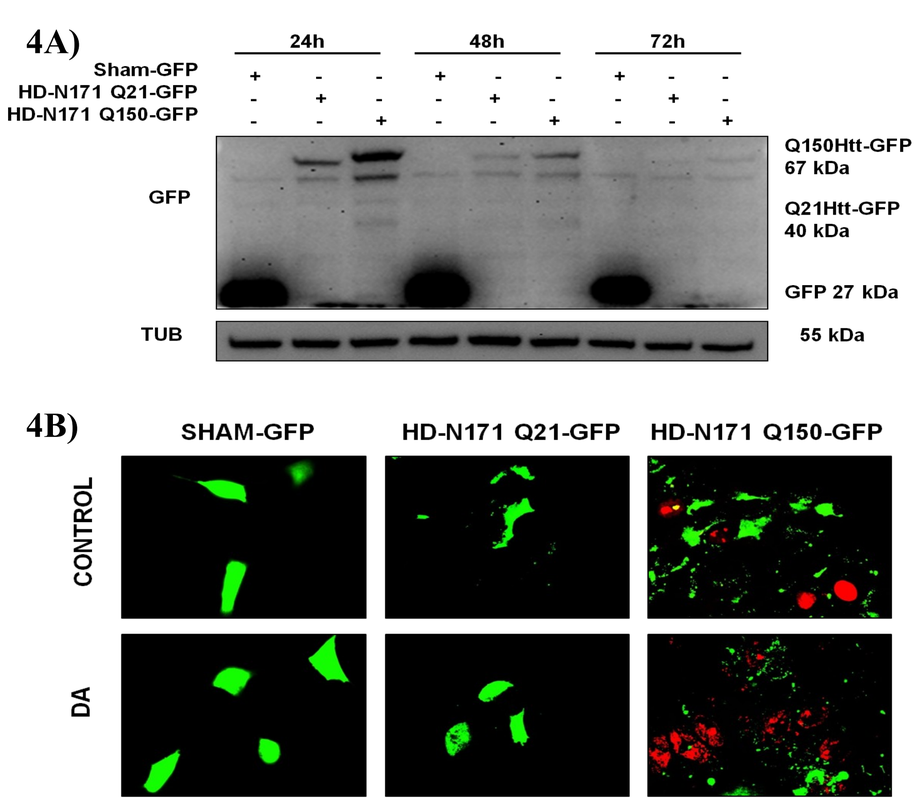



**Fig. 4A**: Cells transfected with Htt N-terminal fragments, HD-N171 Q21 and HD-N171 Q150, display a peak of Htt expression at 24h, which is maintained until 48h and then significantly decreases at 72h.

**Fig 4B,C**: The DA-induced necrotic toxicity is exacerbated in HD-N171 Q150 expressing cells after 24h treatement; accordingly, the PI staining is higher in this condition compared to control.

Further, z-VAD-fmk treatment partially prevents necrotic cell death induced by DA.

### DOPAMINE induces necrosis in HD-N171 Q150 expressing neuroblastoma cells




### Inhibition of Autophagy sensitizes to DOPAMINE in HD-N171 Q150 expressing cells

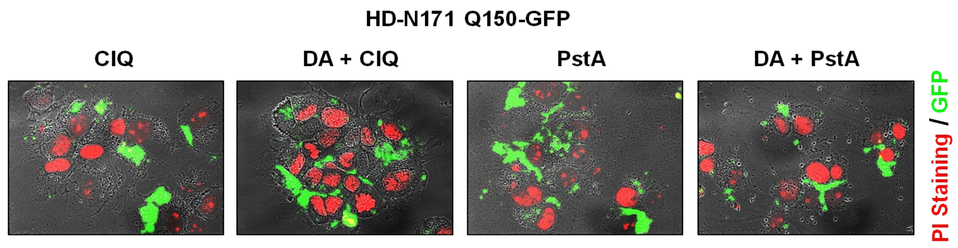



**Fig 5**: The autophagic inhibitors ClQ and PstA exacerbate DA toxicity.

## CONCLUSIONS

Our preliminary data indicate that oxidative stress by DA exacerbates the toxicity of transgenic mutant Htt and suggest the involvement of the autophagy-lysosomal system and of Cathepsin D-mediated proteolysis in the clearance of Htt and in the toxic effects.

**REFERENCES**

**1**- Cagnin et al., Brain Res 2012 **2**- Chen et al., Front Neurosci 2013 **3**- DiFiglia et al., Science 1997 **4**- Fulda et al., et al., Int J Cell Biol. 2010 **5**- Liang et al., Mol Neurobiol 2011 **6**- Qin et al., Hum Mol Gen 2009 **7**- Rubinzstein, Trends Genet 2002