Development of a Cellular Assay for Huntingtin's Pro-Survival Function

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Introduction

Huntington's disease (HD) is an autosomal dominant neurogenerative disorder caused by the expansion of a CAG trinucleotide repeat within the N-terminus of the huntingtin gene. This mutation causes a toxic gain of function in the protein huntingtin leading to the death of spiny neurons in the striatum. Main deficits entailed in HD include cognitive decline with decreases in attention and mental functions, alongside progressive severe motor impairments.

Wild-type huntingtin function is required for normal neurodevelopment and the loss of expression results in embryonic lethality, while less than half loss of expression has been evidenced to display neuropathological symptoms. Huntingtin has pro-survival effects in several model systems, but the mechanisms involved are not well understood.

Our study aims to develop **a cellular assay of huntingtin function** utilizing anoikis, a specific form of apoptosis important in oncogenesis. **Anoikis** results through loss of attachment, such as to the extracellular matrix and will be utilized to characterize the pro-survival function of huntingtin.

The development of an *in vitro* model for the pro-survival function of huntingtin will allow us to dissect critical steps involved in this important neurodevelopmental process.

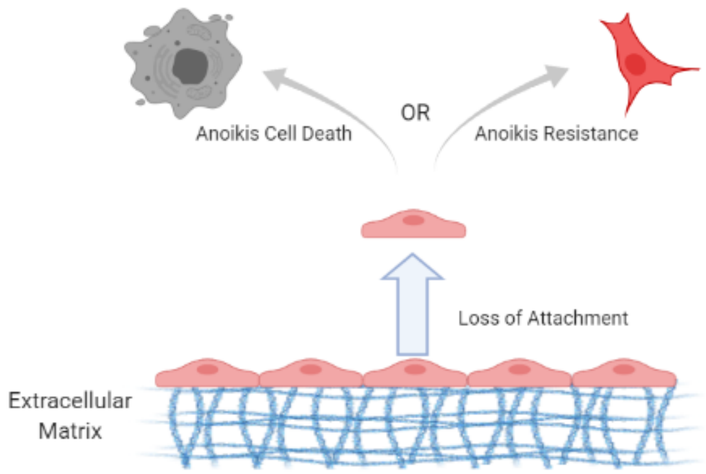

Figure 1. Anoikis example in endothelial cells caused by loss of attachment to the extracellular matrix leading to a programmed cell death in to prevent abnormal cell growth or attachments. Anoikis resistance may be acquired through activation of pro-survival pathways. Acquisition of such mechanisms are most characterized in cancer cells, as resistance is a requirement for metastatic movement.

Hypothesis

We hypothesize that altering the pro-survival function of huntingtin will modulate cell death in anoikis.

Methods and Results

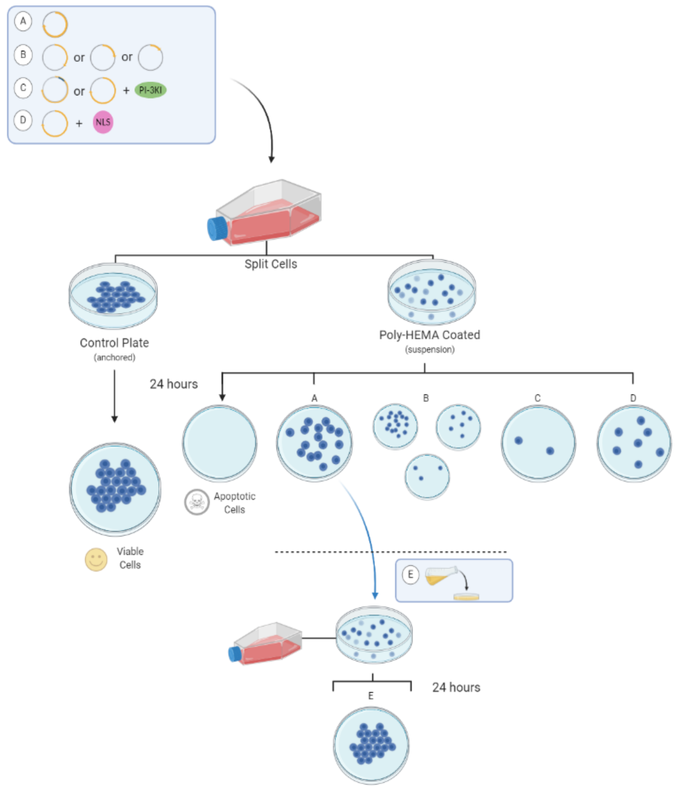

Figure 2. Pro-survival assay development experiments demonstration using epithelial cell line HBL-100 with controls and comparisons using a poly-HEMA coated plate as a loss of attachment condition to measure anoikis resistance, 24-hour incubation time and cell death measured through annexin 5 staining. Treatment options depicted are the following: A- transfection of a 3949-nucleotide long huntingtin construct (**Figure 3**); B- transfection of huntingtin constructs of various lengths: 1955, 1597, 989 (**Figure 4**) C- transfection of huntingtin construct containing S421 mutation to A or 3949- huntingtin construct with a PI-3 kinase inhibitor **(Figure 5 & 6).** D- transfection of a 3949-huntingtin construct with a nuclear localization signal **(Figure 7).** E- non transfected cultured with the conditioned media of cells that have been transfected with 3949- huntingtin (**Figure 8**).

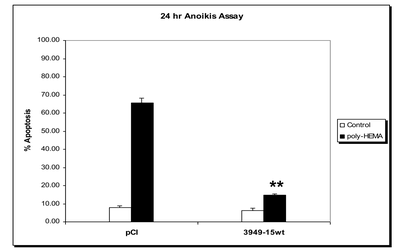

Figure 3. Cell death assay using annexin 5 staining comparing transfection of a control construct pCI-neo and a 3949-nucleotide long huntingtin. Indication of a pro-survival effect with a huntingtin construct. **p<0.01

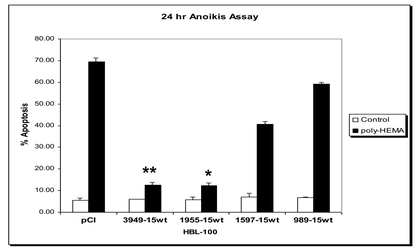

Figure 4. Cell death assay using annexin 5 staining comparing transfection of a control construct pCI-neo and differing lengths of huntingtin construct: 3949, 1955, 1597, and 989 nucleotides. Pro-survival effects in cells with a >1955 huntingtin construct. *p<0.01, **p<0.001

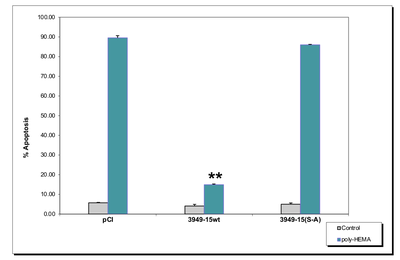

Figure 5. Cell death assay using annexin 5 staining comparing transfection of controls and a 3949-huntingtin construct containing a mutation of S421 to alanine. Decreased pro-survival effect in the absence of a phosphorylated serine. **p<0.001

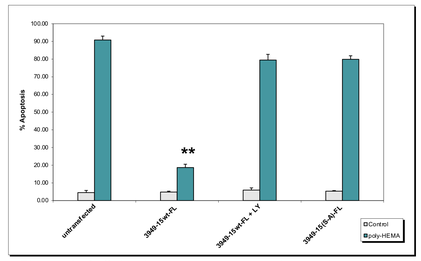

Figure 6. Cell death assay using annexin 5 staining comparing transfection of controls and a 3949-huntingtin construct with LY294002 (PI-3 Kinase Inhibitor). Decreased pro-survival when you inhibit Akt. ** p<0.001

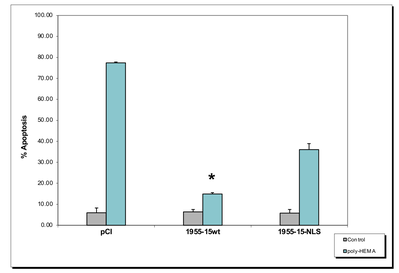

Figure 7. Cell death assay using annexin 5 staining comparing transfection of controls and a 1955-huntingtin construct with a nuclear localization signal. Decreased pro-survival effect in the nucleus. *p<0.01

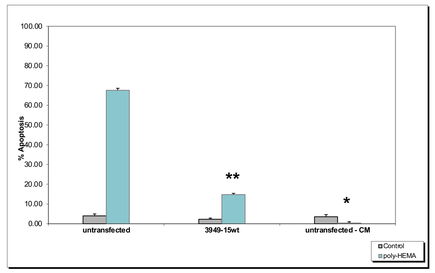

Figure 8. Cell death assay using annexin 5 staining comparing transfection of controls and non transfected cells using conditioned media from 3949-huntingtin construct transfected cells. Transfer of pro-survival effect through conditioned media. *p<0.001

Conclusions and Future Directions

- There is a pro-survival effect with transfection of a wild-type huntingtin as indicated through decreased cell death via anoikis resistance.
- The first 1955 nucleotides of huntingtin are sufficient and required for pro-survival likely mediated via huntingtin phosphorylation by Akt at serine 421.
- Cytoplasmic localization is required for a full pro-survival effect.
- Huntingtin appears to secrete soluble factor that can relay the pro-survival effects.

These experiments will be reproduced, extended and validated using a human umbilical vein endothelial cell line, previously been evidenced to exhibit anoikis in similar conditions as the previously used epithelial cell line HBL-100. These findings will continue to inform us about the mysteries of wild-type huntingtin function and can have implications for non-specific huntingtin lowering HD treatments.

Acknowledgements