Engineering an Protein Aggregate Model to Study Autophagy Pathways in Human Cells

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Introduction

Neurodegenerative diseases such as Parkinson's and ALS are marked by the deposition of protein aggregates in cells. Cells have control mechanism that they have evolved to use, and that is a selective subtype of autophagy called aggrephagy which helps to clear out these toxic aggregates and maintain proteostasis. This study establishes a fibroblast model using an inducible system to express aggregation-prone protein, such as α-synuclein, FUS, and TDP-43, and find out whether fibroblasts utilize autophagy pathways to degrade these pathological clumps. By using primary human fibroblasts and visualizing aggregate formation through aggresomal staining. This model enables the study of clearance dynamics in a cellular context giving insight into basic cellular control mechanisms against misfolded protein stress (Mizushima, 2007).

Objectives

- Establish that transgenes of aggregate forming proteins do form aggregates in human fibroblasts
- Transduce human fibroblasts with an inducible promotor system
- Transduce human fibroblasts with an inducible transgene expressing aggregate forming proteins

Methods

1. Cell Source:
   Human skin fibroblasts from an 18-year-old donor served as the cell source
2. Transduction of Lentiviral:
   Using lentivirus and polybrene, the Tet-On 3G system—which contains the hygromycin resistance gene—was introduced. After 24 hours, the media switched to FBS TET-free to avoid leaky expression.
3. Optimisation of Antibiotic selection:
   Different hygromycin dosages were administered to cells (post-24–48h transduction). Cell survival is monitored to determine the ideal viral volume and antibiotic concentration.
4. Aggregation-Prone Protein Expression:
   Halo-GFP-tagged α-synuclein, FUS, and TDP-43 mutants were utilised for the second transduction. Aggregate formation is triggered by doxycycline induction
5. Identification of aggresomes:
   Post induction, an aggresome detection kit is used. Visualization via fluorescence microscopy. (Hanada et al., 2007).

Results

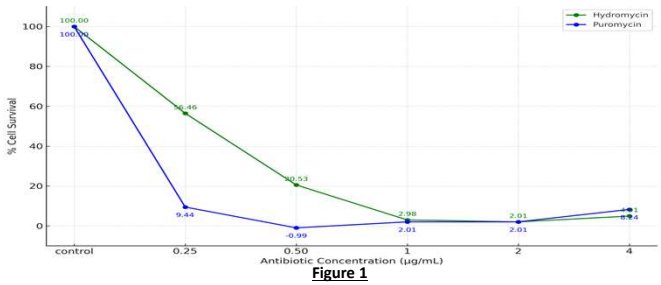


Figure 1

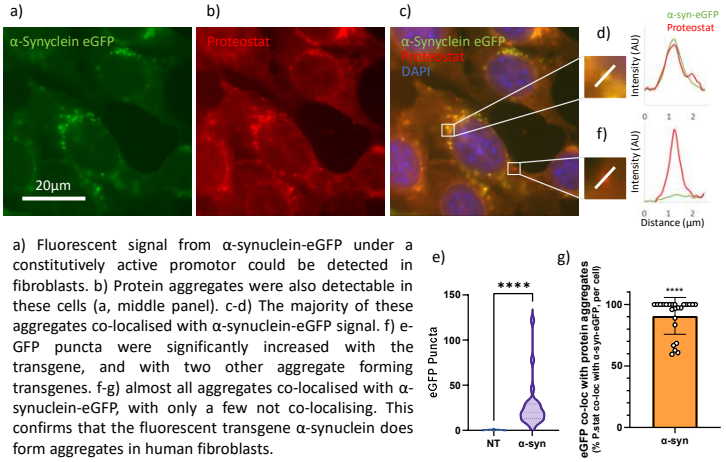
(Antibiotic Kill Curve in Human Fibroblasts (Hygromycin vs. Puromycin)

Hygromycin showed a dose-dependent drop in cell survival, from around 100% at control to about 20% at 0.5 µg/mL and <5% between 1–2 µg/mL. This suggests that 0.5–1 µg/mL is the ideal concentration for choosing effectively transduced cells while maintaining a small surviving population (Sigma-Aldrich, 2022).

At 0.25 µg/mL, puromycin led to nearly complete cell death, demonstrating how sensitive fibroblasts are to the antibiotic. The inability to identify a safe selection window suggests that it is unsuitable for this sort of cell (Kill curve standards, 2011) .

According to established protocols, these results support the idea that each cell line and antibiotic should have its kill curve reviewed in order to identify the lowest selection dose that minimises cytotoxic stress while killing >99% of non-transduced cells.

α-synuclein-eGFP forms aggregates in human fibroblasts



a) Fluorescent signal from α-synuclein-eGFP under a constitutively active promotor could be detected in fibroblasts. b) Protein aggregates were also detectable in these cells (a, middle panel). c-d) The majority of these aggregates co-localised with α-synuclein-eGFP signal. f) e-GFP puncta were significantly increased with the transgene, and with two other aggregate forming transgenes. f-g) almost all aggregates co-localised with α-synuclein-eGFP, with only a few not co-localising. This confirms that the fluorescent transgene α-synuclein does form aggregates in human fibroblasts.

TET-On inducible α-synuclein-eGFP forms aggregates in human fibroblasts when activated by Doxycycline

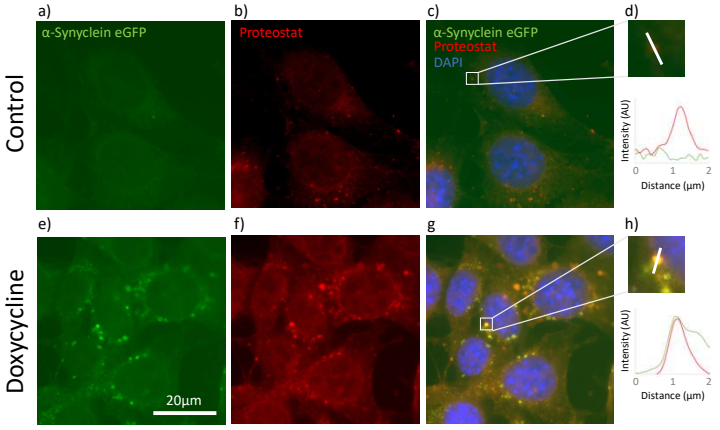


Figure legend goes here. Doxycline conc was 100ng/ml
Essentially no foci in control cells and low level of proteostat staining
The occasional foci may be due to very minor 'leaky expression'
Lots of foci when cultured for 72 hours in Doxycyclilne.

Conclusion

Through this project, we developed a fibroblast model that can be switched on to produce proteins that are prone to forming aggregates which is a key feature of neurodegenerative diseases. Using a viral delivery and antibiotic selection, we established stable cells and showed that doxycycline induction leads to clear aggregate formation which was confirmed by staining. This model provides a straightforward framework to study how cells are able to clear toxic clumps and sets the stage for future tests of autophagy & gain more understanding of the cellular pathways and potentially discovering novel therapeutic approaches that can help in people suffering from neurodegenerative disease

References :
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