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A Cellular Model adapted to High Content Drug Screening for Parkinson´s Disease

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Abstract

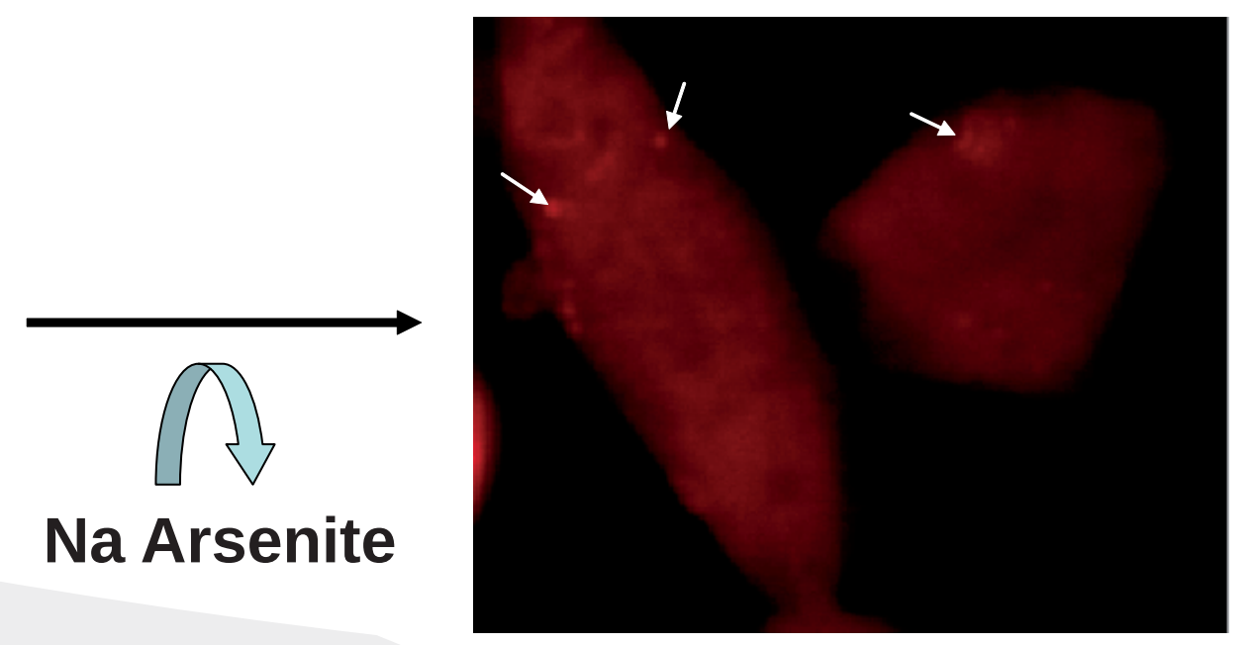
Alpfa-synuclein or SNCA is a synuclein protein that is found predominantly in the presinaptic termini of neurons. Aggregates of SNCA, which are toxic to the cell, are the main structural component of Lewy bodies. This confers to the protein a central role in the pathology of Parkinson´s Disease (PD) and other neurodegenerative disorders. The wild-type SNCA itself can form unsoluble aggregates; however, some mutations of the gene coding for alpha-synuclein or a multiplication of its locus can be the cause of some forms of the hereditary Parkinson Disease. It is known also that unsoluble SNCA can be released to extracelular medium and become toxic for other healthy neurons, spreading this way the pathogenic SNCA and the disease. Our company has developed a cell-based model for the screening of inhibitors of the transmission between culture cells of unsoluble SNCA in order to be tested in HCS assays.

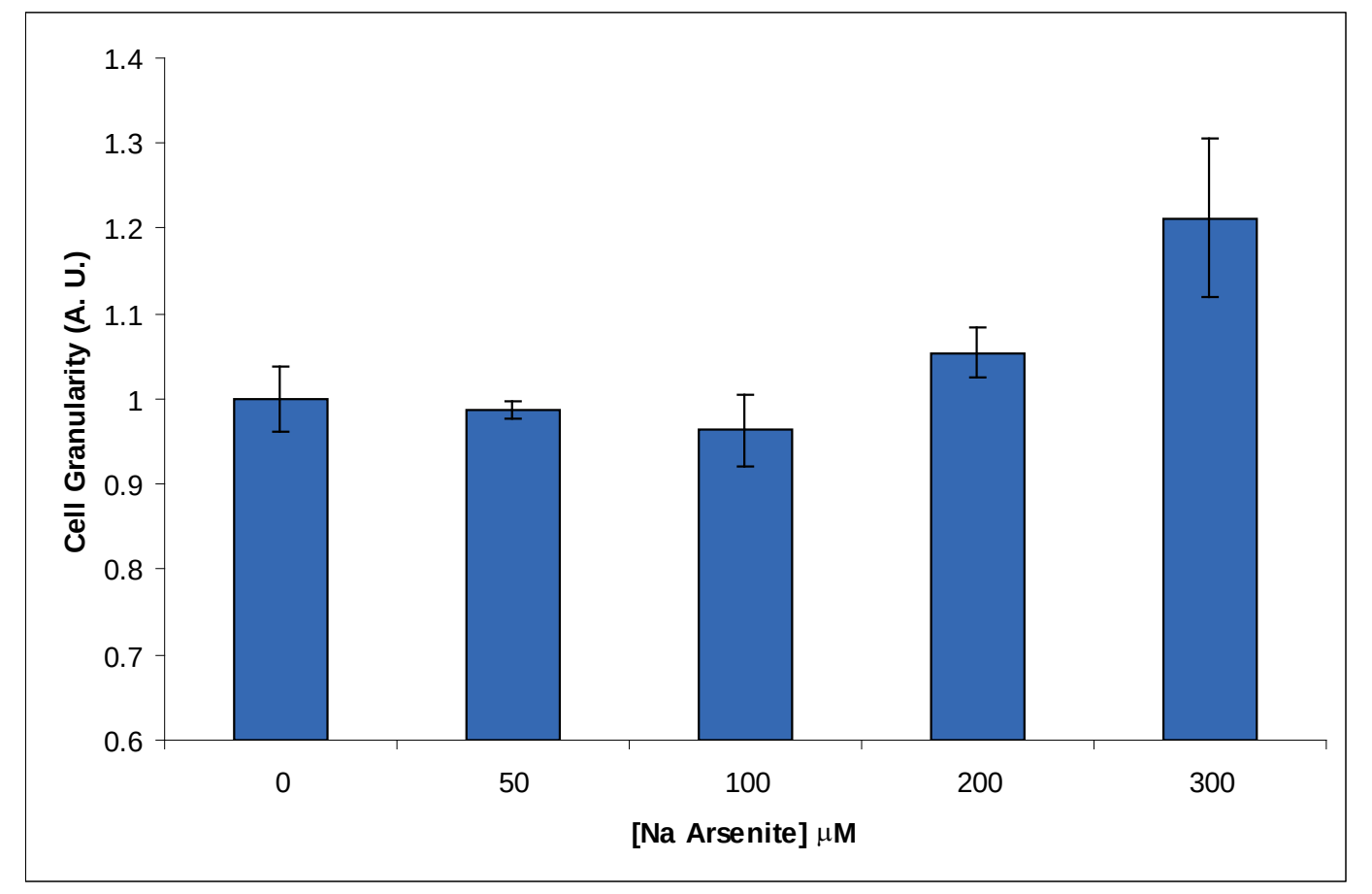
Results

The finding that α-synuclein aggregates are present not only in the cytoplasm of cells, but also in CSF and blood plasma of PD patients, lead us to develop different cellular models to study new possible drugs against this disease.

A) Aggregation of α-syn in the cytoplasm of culture cells :

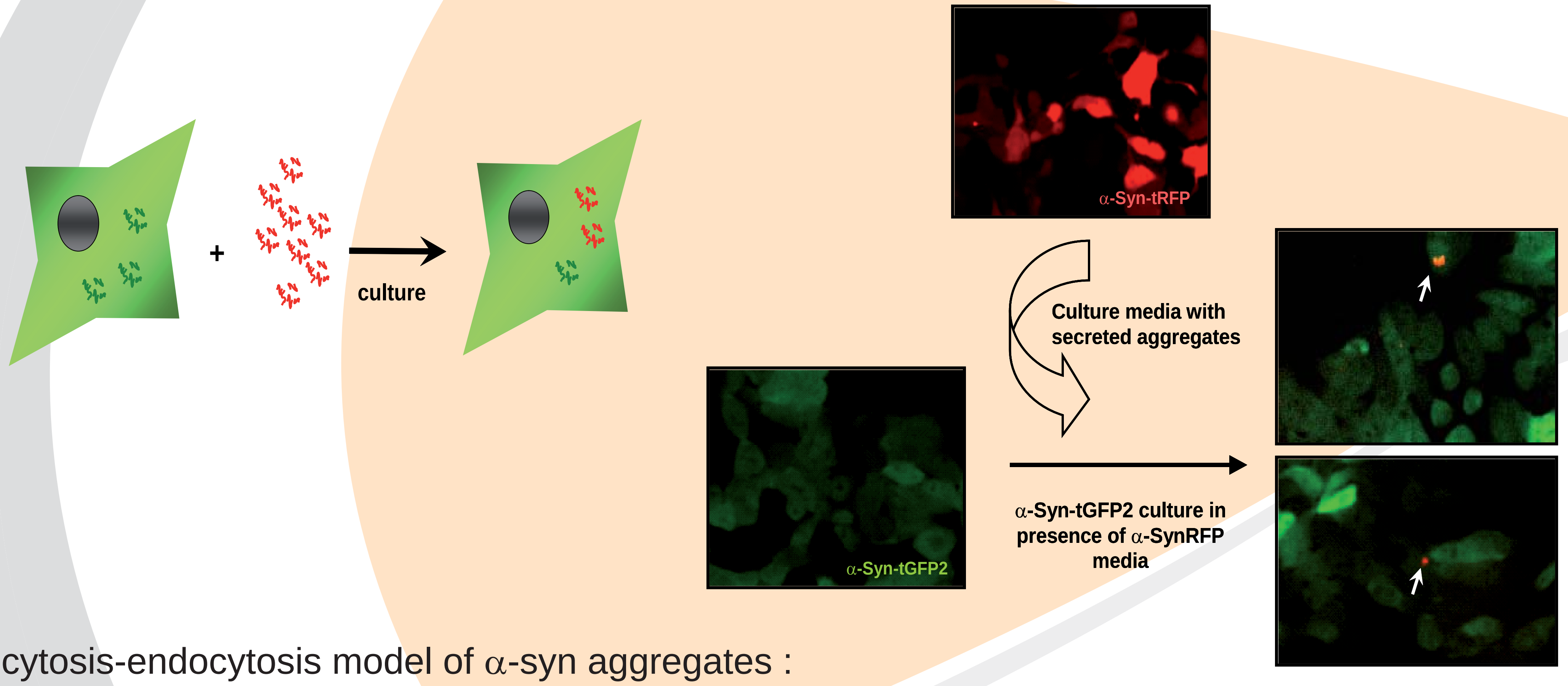
The aggregates of α-syn tagged with tagRFP (Evrogen) were produced by the addition of different concentractions of sodium arsenite into stably expressing SH-SY5Y or HEK23 culture cells for 90 minutes. Pathological α-syn-tagRFP aggregates were quantified with a BD Pathway 855 image platform.





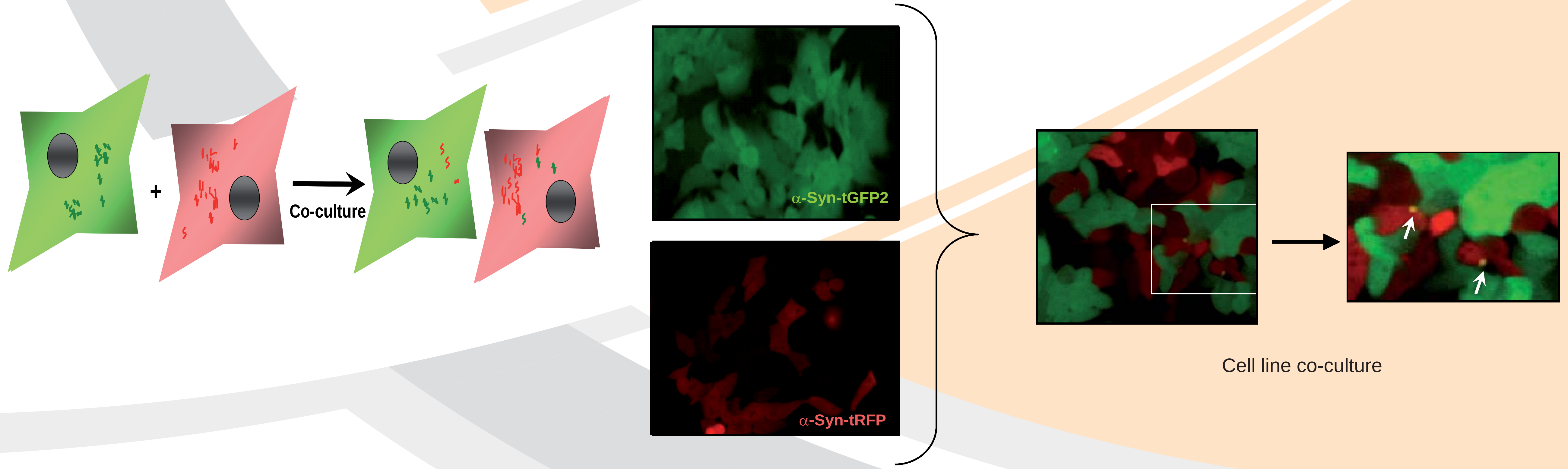
B) Endocytosis model of α-syn aggregates :

First, the aggregates of α-syn tagged with tagRFP were recovered from culture media after being secreted from stably expressing SH-Sy5Y or HEK293 cells. Then, the α-syn-tGFP2 (Evrogen) stable cell line was cultured in presence of α-syn-tagRFP recovered media. The presence of α-syn-tagRFP aggregates into α-syn-tGFP2 cells was observed 24h after treatment by fluorescence pseudoconfocal microscopy.



C) Exocytosis-endocytosis model of α-syn aggregates :

The α-syn-tagRFP and the α-syn-tGFP2 SH-SY5y or HEK293 stable cell lines were co-cultured. The exchange of aggregates were observed 48h after treatment by fluorescence pseudoconfocal microscopy.



Cell line co-culture

Materials and methods

Cultured cells: SH-SY5Y and HEK293 α-synuclein cell lines were cultured into 96 wells Imaging Plates (BD) at 0.25 cells/cm2 in 200 ul of RPMI 10% FBS and DMEM 10% FBS respectively, and incubated at 37ºC and 5% CO2.

Image adquisicion: human tagged α-syn stably expressing cells were treated with sodium arsenite for 90 minutes and with aggregates-media or co-cultured during 24 or 48 hours. After that, the presence of aggregates was quantified by fluorescence using image analysis algorithms and the BD Pathway 855 image platform.

Conclusions

The stably transfected α-syn cells can be used to produce biologically endocitable α-syn aggregates.

The α-syn aggregates can be increased in these cells in a quantitative manner by the addition of an oxidative stressing agent.

The stably transfected α-syn cell model can be used for inhibitors discovery against pathological secretion-endocytosis of aggregates.

This model has been adapted to HCS analysis based in image algorithms to test the process of aggregates exchange.

This model permits evaluating the α-syn aggregates in living cells through the study of its location pattern in the space and time.

This model provides a strategy to evaluate drugs against the exchange of aggregates without the need of being permeable.

References

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