Identification of kinetic neurodegeneration events in patient-derived cell models

P1055

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Abstract

The long term microscopy images of live cell fluorescent reporters on patient-derived cell models provide a wealth of information to study the kinetic phenotypes and biomolecular events of neurological disease states. To discover the kinetic phenotypes of disease process embedded in induced motor neurons (iMNs), we developed and optimized a functional reporter Smac-GFP$_{11}$ that signals the cell apoptosis. In a longitudinal imaging study on Molecular Devices ImageXpress, both iMN patient and healthy control lines are imaged with the Smac-GFP$_{11}$ apoptosis and Hb9::RFP motor neuron lineage reporters once every 12 hours for 28 days. We detected and tracked individual iMNs and are able to perform Hb9::RFP and Smac-GFP$_{11}$ co-expression analysis.

Apoptosis Indicator: Smac-GFP-OPT

We developed an apoptosis indicator- Smac-GFP-OPT using the probe pair of Smac-GFP11 and GFP1-10 (Figure 1 A). We verified the color separation and localization using Hela cells irradiated by UV. Under apoptotic condition in HeLa cell, we confirmed the Smac-GFP11 is released from mitochondria into cytosol. It is then spontaneously complemented with cytosolic GFP1−10 (Figure 1B), resulting in the recovery of the fluorescence. Figure 1C shows the temporal variation of fluorescence in the iMN cell region .

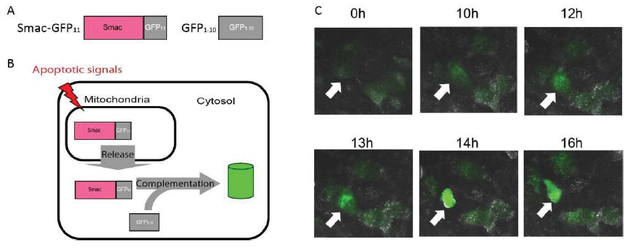


Figure 1. Schematic of Smac-GFP-OPT

iMN Detection using ML-powered Classifier

A Machine Learning-powered classifier is used to detect iMNs in HB9::RFP channel. The classifier was pre-trained using the positive and negative iMN examples labeled by experts in HB9 training data. When applying the pre-trained classifier in HB9::RFP channel, the iMN-alike objects are first detected as potential objects (Figure 2, top) scoring from 0 to 1 based on their morphological features of soma (green in Figure 2, bottom) and neurites (pink in Figure 2, bottom). The objects scoring above a threshold of 0.5 are segmented as iMNs (cyan in Figure 2, top).

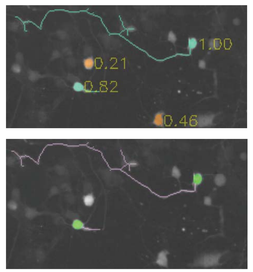


Figure 2. iMN detection in HB9::RFP

Longitudinal iMN Tracking

After segmenting the iMN cell bodies in HB9::RFP, we applied the cell tracking recipe of Aivia 7.7 to track the lifetime of iMNs soma for 15 days (30 frames). In addition, the mean intensity of soma in Smac-GFP channel is measured to determine the GFP co-expression state (Figure 3). The iMNs successfully tracked for longer than 10 frames (108 Hours) are selected for categorization:

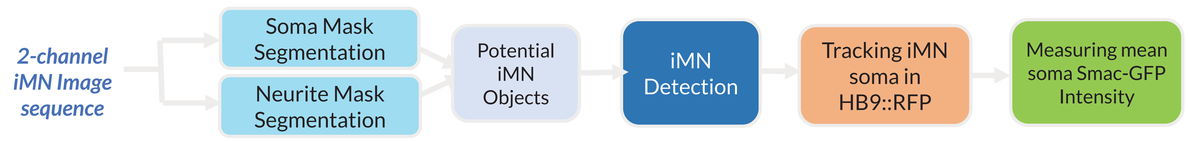


Figure 3. Flow chart of iMN detection, tracking, and measurements

The iMNs tracked until the end of 30-frame movie are categorized as “neuron survival”, otherwise categorized as “neuron death”; the iMNs are categorized as “co-expression” if its mean soma Smac intensity is 10 (max = 255) counts greater than the Smac-GFP background intensity for at least 6 frames, otherwise categorized as “non co-expression”. Figure 4 shows examples of the four categories.

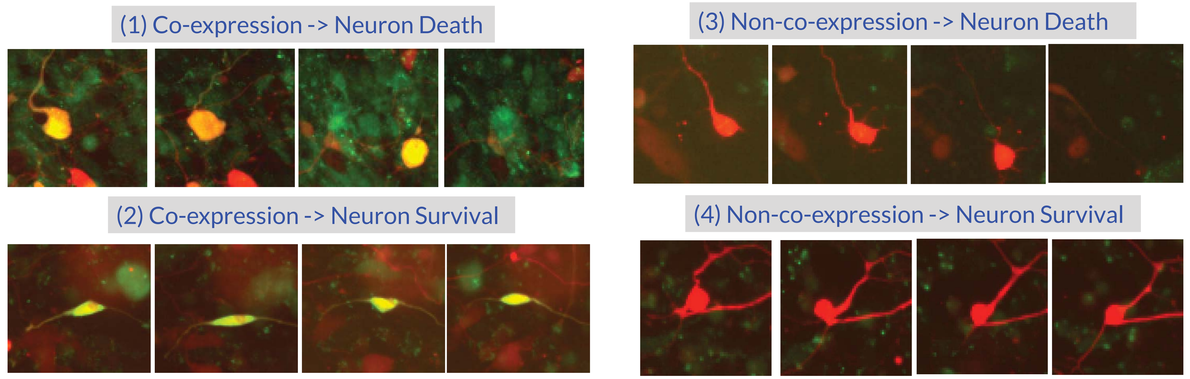
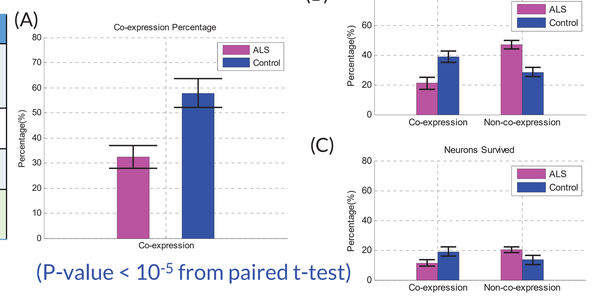


Figure 4. iMN examples of four categories: Co-expression ➔ (1) Neuron death, (2) Neuron survival; Non-co-expression ➔ (3) Neuron death, (4) Neuron survival

Co-expression and Neurodegeneration Analysis

iMN Categories	ALS		Control	
	Co-expression	Non-Co-expression	Co-expression	Non-Co-expression
Neuron Death	21.14%	47.16%	38.88%	28.69%
Neuron Survived	11.34%	20.36%	18.94%	13.49%
Total Co-expression/ Non-co-expression	32.48%	67.52%	57.82%	42.18%

Table 1. The mean percentages of different categories of iMNs from 6 replicate wells of ALS patient line and Healthy Control.
(total ALS iMNs= 1,830, total Control iMNs = 2,882)



(P-value < 10^{-5} from paired t-test)

Figure 5. Comparison of ALS versus control of A) co-expression percentages, B) neuron death, and C) neuron survival rates.

We tracked the iMNs during Day 13 –27 (15 days, 30 frames) in survival image sequences acquired from one ALS patient panel and one healthy control. We analyzed 6 replicate wells for both cases, and the mean/standard deviation for the percentages of 4 categories are presented in Table 1 and Figure 5. The observed “Co-expression” percentage in Control is statistically significantly higher than ALS in both “Death” or “Survival” categories given their similar survival rates. This suggests that a different pathway may be inducing neuron death in ALS patient as compared to the healthy control.

Conclusions and Future Work

We characterized four different kinetic co-expression event types, and the differences in event type prevalence could lead to different neurodegeneration outcomes. We are developing and optimizing three additional cell functional fluorescent reporters for human patient lines. The additional reporters could further elucidate kinetic phenotypes of neurological disease states.

Acknowledgements

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