Survival analysis of human in vitro-derived neurons using new live cell extended time-lapse imaging technology

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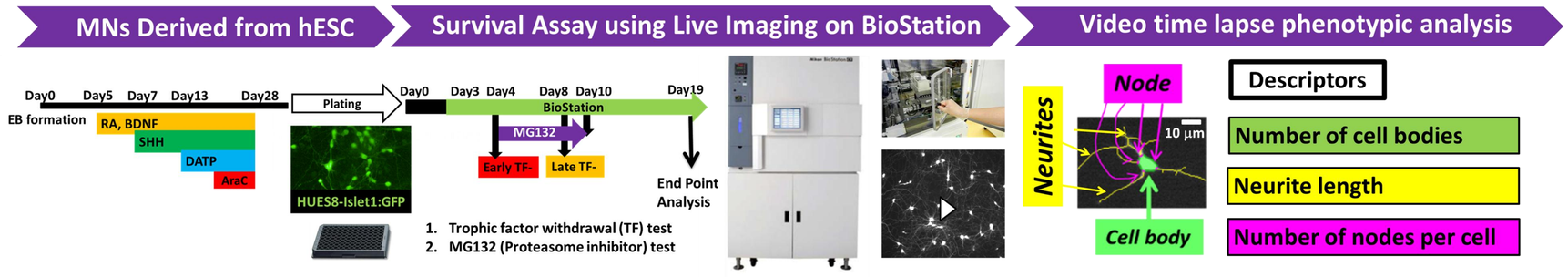
Summary

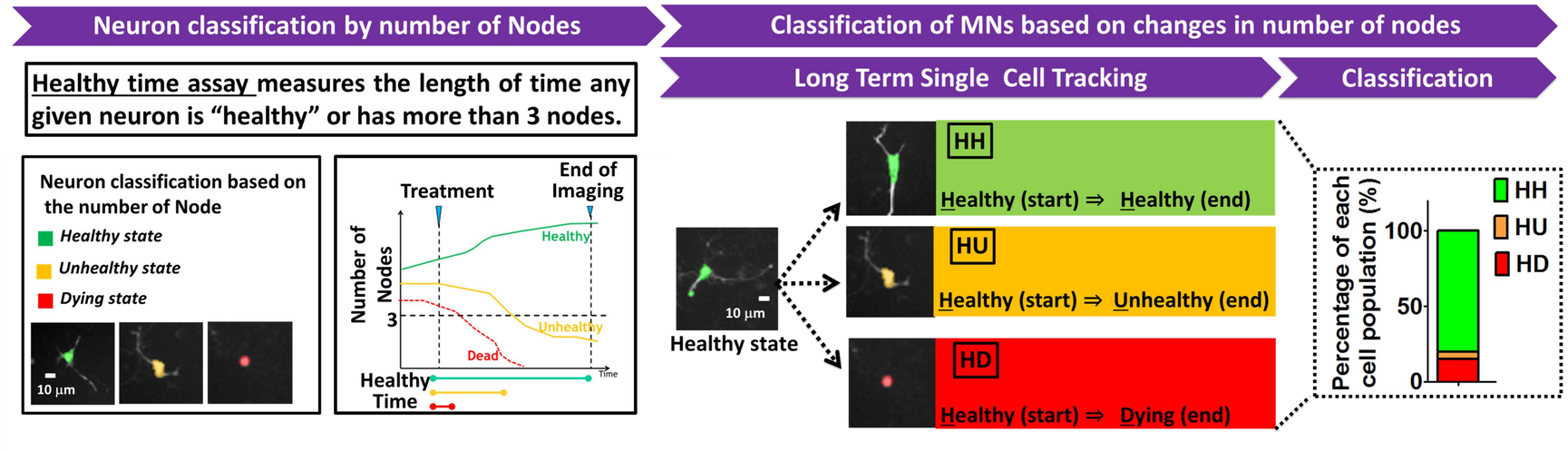
Long-term live cell imaging of stem cell-derived neurons has the potential to be a novel platform for drug discovery in neurodegenerative disease which has not been successfully implemented. We have conducted detailed long-term live analysis of stem cell-derived motor neurons (MNs) using the Nikon BioStation CT. Imaging analysis algorithms accurately tracked key attributes such as cell body size, neurite number and neurite length. We used these imaging tools to study MNs subjected to two different stressors: neurotrophic factor (TF) withdrawal at early and late times and treatment with the proteasome inhibitor MG132. We have defined a new, morphological predictor for cell death that involves measurement of neuritic changes. This analysis algorithm determines the "healthy time (HT)" for each MN, and this metric reveals the kinetics of survival responses that are not apparent with endpoint analysis. Quantifying disrupted neurites as an index of disease onset is advantageous since these changes occur early in the death process. These studies indicate that live cell imaging is a useful platform for characterizing stem cell-derived populations and may be a novel approach to identifying new therapeutic molecules.

Purpose

- To overcome the limitations in endpoint immunostaining analysis, we sought to create a live cell imaging platform to characterize survival responses of in vitro-derived neurons
- Identify morphological characteristics that discern survival of neurons that precede cell death and which can be tracked over time

Methods





Tracking number of nodes and neurite length of Motor Neurons (MNs) is more informative than the number of cell bodies

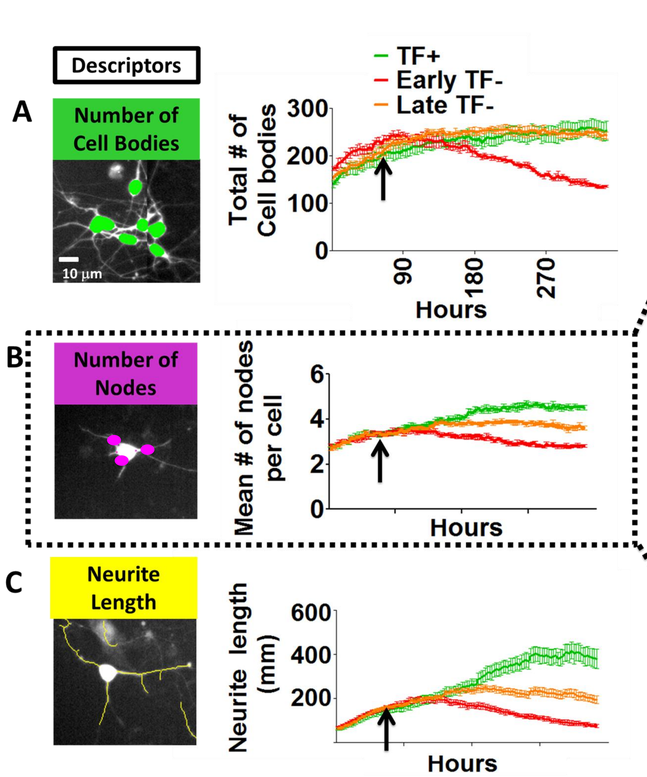


Figure 1. Early trophic factor (TF) withdrawal has a more substantial effect on motor neurons than late TF withdrawal. A. Time plot of MN cell body number over two weeks of imaging B. Time plot of average number of nodes per MN. C. Time plot of total neurite length. (arrow: TF factor withdrawal)

Live single cell tracking of MNs by number of nodes reveals that changes in node number are an early event following cell stress

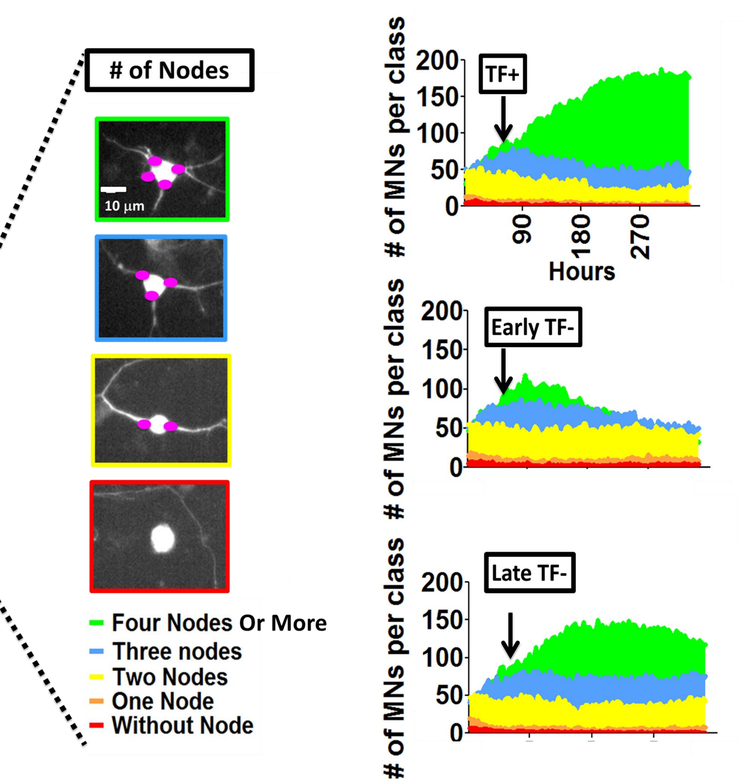


Figure 2. Following early TF-, there is a rapid loss of MN with 4 or more nodes. In late TF-, there is a delayed and more subtle change in the population of MN with 4 or more nodes. (arrow: TF factor withdrawal)

Classification of MNs based on changes in number of nodes provides more detailed survival information than traditional endpoint

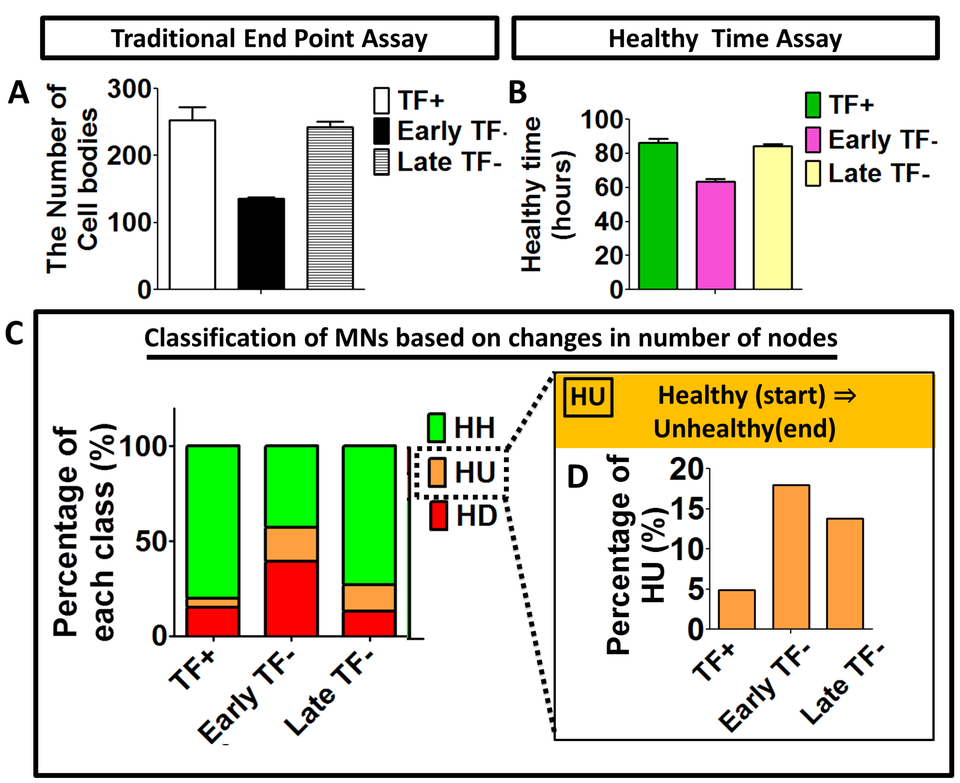


Figure 3. The slow death response of late TF- is apparent with live cell analysis, but not evident by endpoint analysis. A. With endpoint analysis, there is no significant death following late TF -. B. Healthy time measures the time during which MNs have 3 or more nodes. C. Categorizing outcome of neurons with 3 or more nodes shows the changes in distribution of healthy and unhealthy MN. D. The HU population is increased following early and late TF-.

Conclusions

- The Nikon BioStation CT can accurately track individual motor neurons derived from ES cells for up to 2 weeks in either 48 well or 96 well plates
- Characterizing motor neurons by changes in node number captures more detailed population information than traditional measurements
- In vitro-derived MNs have a differential response to TF withdrawal, depending on the time at which the stress is given
- Live single cell imaging has the potential to add new dimensions to characterizing human diseases mechanisms using iPS cells

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