Abstract

Direct conversion of fibroblasts derived from amyotrophic lateral sclerosis (ALS) patients using a combination of seven transcription factors [1] has been used to produce induced motor neurons (iMNs) that recapitulate the disease phenotype. However, latency in the conversion process produces a mix of iMNs and fibroblasts with similar morphology. To screen for treatment compounds, there is a critical need for an automated analytical tool capable of distinguishing between these populations algorithmically to identify desired phenotypes in patient-derived cells.

We have developed a machine learning-enabled analytical tool to detect ALS patient-derived iMNs and track their survival rates over time. Machine learning-enabled classifiers are trained on iMNs and are used to reject false positives and to detect bona fide iMNs. We applied the tool to a 300-compound screen to measure long-term survival rates for iMNs.

Direct Conversion Protocol

1. Extract fibroblasts
2. Add transcription factor
3. Add compounds

Neurite Segmentation

Enhance filaments
Segment objects
Enhance blobs
Segment objects

Figure 3. Multiscale vessel enhancement filter [2] is used to enhance cell body and neurites separately. The green arrowheads indicate the enhanced features while red arrowheads indicate suppressed features. Objects are segmented by threshold on both enhanced images and combined in the final step to generate the combined iMN detection (orange overlay). Scale bar: 100 µm.

Survival Analysis

Figure 5. Fibroblasts (yellow arrowhead) were converted into iMNs (green asterisk) throughout the survival study. Over time, neurites began to shrink (red arrowhead) in iMNs preceding cell death (red asterisk). Some iMNs incubated with PIKfyve inhibitor showed continued neurite growth (green arrowhead) after 14 days. Scale bar: 500 µm.

Summary

- We developed a machine-learning based analytical tool for tracking long-term survival of iMNs.
- iMNs incubated with a neuroprotective small molecule inhibitor shows increased survival rate.
- The tool, combined with long-term imaging, provides a scalable platform that could complement RNA profiling for drug screening in ALS patients.
- We plan to apply the tool to a 4,000-compound pilot screen, followed by a 40,000-compound screen.

References


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