Title
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Journal
Science Translational Medicine, 5(188)

ISSN
1946-6234

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Publication Date
2013-06-05

DOI
10.1126/scitranslmed.3005065

Peer reviewed

STEM CELLS

Comment on "Drug Screening for ALS Using Patient-Specific Induced Pluripotent Stem Cells"

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Egawa *et al.* recently showed the value of patient-specific induced pluripotent stem cells (iPSCs) for modeling amyotrophic lateral sclerosis in vitro. Their study and our work highlight the need for complementary assays to detect small, but potentially important, phenotypic differences between control iPSC lines and those carrying disease mutations.

In their recent study, Egawa *et al.* (*1*) analyze motor neurons differentiated from induced pluripotent stem cell (iPSC) lines derived from patients with amyotrophic lateral sclerosis (ALS) carrying several different mutations in TDP-43 (*1*). Using multiple mutant TDP-43 iPSC lines, they confirm and extend in detail the in vitro recapitulation of ALS-associated phenotypes that we had shown previously (*2*). In contrast to our findings (*2*), Egawa *et al.* state that they do not observe a survival difference between cultured motor neurons derived from mutant TDP-43 iPSC lines and control iPSC lines under basal conditions when using a lactate dehydrogenase (LDH) release assay to measure neuronal survival [fig. S11C in (*1*)]. In our study (*2*), we did not make any claims about the survival of iPSC-derived mutant ALS and control motor neurons under basal conditions measured using the LDH release assay. However, we did report a survival difference when using the LDH release assay in the presence of a stressor [Fig. 5C in (*2*)] and also when using real-time single-cell longitudinal survival analysis under basal conditions [Fig. 5B in (*2*)]. We would like to clarify this issue as well as discuss the value of using complementary survival assays.

Specifically, in our study, we aimed to address two issues regarding the effect of the M337V TDP-43 mutation on motor neuron survival (*2*). To address whether the M337V mutation caused an inherent neuronal vulnerability under basal conditions, we used real-time single-cell longitudinal image analysis in which only the neurons that expressed a fluorescent reporter construct on the first day of the assay were followed serially and monitored for survival over 10 days [Fig. 5B in (*2*)]. Next, we investigated at the population level whether the TDP-43 mutation conferred a selective sensitivity to a stressor, which was assessed by a fluorometric cytotoxicity assay that measured LDH enzyme released into the culture medium by neurons that had lost membrane integrity [Fig. 5C in (*2*)]. Given the dynamic nature of iPSC-derived neuronal populations, a certain level of cell death and limited proliferation of residual neural progenitors are expected, and this background activity can potentially mask subtle differences in survival. In fact, a breakdown of our control LDH release assay data (Fig. 1A, this Letter) does not reveal a difference in survival between M337V mutant and control iPSC–derived motor neurons under basal conditions, which is in agreement with Egawa *et al.*'s results [fig. S11C in (*1*)].

The challenge of elucidating subtle but significant phenotypes in long-term neuronal cultures requires the application of multiple complementary readouts. Real-time single-cell longitudinal survival analysis using fluorescent reporter genes has enabled determination of differences in neuronal survival that may otherwise have been missed using conventional population-based assays such as the LDH release assay (*3*, *4*). Another method to determine survival differences between different populations is to count cells that are positive for a particular reporter/marker at discrete time points. Survival analysis performed by fluorescent reporter–based "snapshot" cell counts at fixed time points can also present challenges of interpretation because upon transient transfection, fluorescent reporters can be expressed stochastically over the time course of the experiment. To test this possibility, we counted daily for 9 days the number of green fluorescent protein (GFP)–positive cultured neurons derived from control iPSCs transfected with a motor neuron–specific HB9::GFP reporter construct. We used this approach instead of performing real-time survival analysis, in which we only followed individual neurons that were GFP-positive on day 1 and recorded their time of death [Fig. 5B in (*2*)]. Our analysis revealed that the total number of GFP-positive motor neurons fluctuated over the course of 9 days under basal conditions and did not differ significantly from day 1 (Fig. 1B, this Letter). However, real-time single-cell longitudinal survival analysis using the same experimental setup did reveal an increased risk of neuronal death in control iPSC–derived motor neurons over time [Fig. 5B in (*2*)].

Rapid developments in iPSC technology now enable the comparison of cellular phenotypes between iPSC lines carrying a mutation

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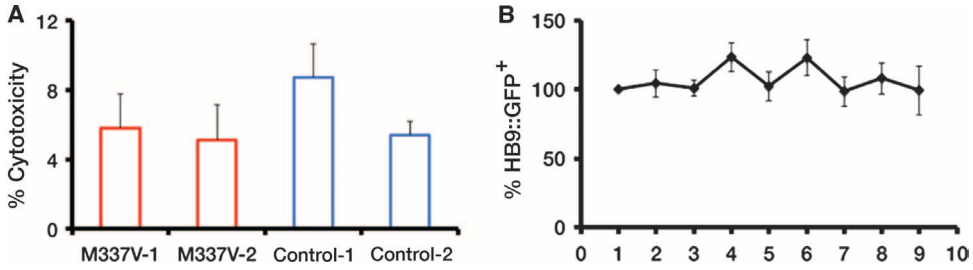

Fig. 1. Survival of ALS mutant versus control iPSC–derived motor neurons. (**A**) Comparison of cell death in motor neuronal cultures derived from two clones of mutant M337V TDP-43 iPSCs from one ALS patient (M337V-1 and M337V-2) and two control iPSC lines (from two different individuals; Control-1 and Control-2) under basal conditions using an LDH release assay (*2*). LDH release into the culture medium was normalized to total LDH after cell lysis for each well to determine percent cytotoxicity. Values are means ± SEM. Data were analyzed by one-way analysis of variance (ANOVA). There were no significant differences between the mean survival for motor neurons derived from mutant M337V TDP-43 iPSCs and control iPSCs (n = 4). (**B**) Cell survival of two independent control iPSC–derived motor neuronal cultures transfected with an HB9::GFP reporter construct. Cell survival was measured by counting the number of GFP-positive neurons over the course of 9 days (*2*). The cell count on day 1 for each experiment was set to 100%, and each time point after that was expressed as a percent of the value at day 1. Values are means ± SEM (n = 4). There were no significant differences in mean survival between day 1 and day 9.

and control lines that do not. Real-time single-cell longitudinal survival analysis is a sensitive method for detecting phenotypes that may otherwise be masked due to variability arising from static measurements. The utility of this approach has recently been confirmed in Huntington's disease patient–derived iPSC neuronal cultures (*5*). This study revealed a higher cumulative risk of death in neurons derived from iPSCs with CAG repeat expansions compared to control iPSCs under basal conditions (*5*). We agree with Egawa *et al.* regarding the importance of independent multiple clonal line–based confirmation of phenotypic differences identified for any given disease mutation. Indeed, such studies would also benefit from the use of complementary sensitive assays to identify potentially important survival phenotypes.

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Submitted 10 January 2013
Accepted 15 May 2013
Published 5 June 2013
10.1126/scitranslmed.3005065

Citation: B. Bilican, A. Serio, S. J. Barmada, A. L. Nishimura, G. J. Sullivan, M. Carrasco, H. P. Phatnani, C. A. Puddifoot, D. Story, J. Fletcher, I.-H. Park, B. A. Friedman, G. Q. Daley, D. J. A. Wyllie, G. E. Hardingham, I. Wilmut, S. Finkbeiner, T. Maniatis, C. E. Shaw, S. Chandran, Comment on "Drug screening for ALS using patient-specific induced pluripotent stem cells." *Sci. Transl. Med.* **5**, 188le2 (2013).

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Science Translational Medicine **5** (188), 188le2. [doi: 10.1126/scitranslmed.3005065]

Editor's Summary

Science Translational Medicine (print ISSN 1946-6234; online ISSN 1946-6242) is published weekly, except the last week in December, by the American Association for the Advancement of Science, 1200 New York Avenue, NW, Washington, DC 20005.