Response to Comment on “Drug Screening for ALS Using Patient-Specific Induced Pluripotent Stem Cells”

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Our work and the study of Bilican et al. highlight the need for complementary assays to detect subtle phenotypic differences between control and mutant induced pluripotent stem cell lines.

We thank Bilican et al. for their thought-provoking comments and for providing new data about cell survival of motor neurons derived from control and mutant M337V induced pluripotent stem cell (iPSC) lines (1). In their new data presented in Fig. 1A of their Letter (1), Bilican et al. demonstrate that there is no difference in cytotoxicity determined by the lactate dehydrogenase (LDH) release assay between motor neurons derived from mutant M337V iPSC lines and control iPSC lines under basal conditions, which is compatible with our results reported in (2). In their original paper (3), Bilican et al. did not show the data for cytotoxicity of motor neurons derived from mutant M337V and control iPSCs under basal conditions measured by the LDH release assay. We agree with Bilican et al. that it is necessary to examine not only cell survival but also cytotoxicity using a variety of different assays including the LDH release assay, caspase-3 activity, and TUNEL (terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick end labeling) staining under basal conditions and in the presence of stressors.

We understand that the LDH release assay in their original paper [Fig. 5C in (3)] was not measuring cytotoxicity under basal conditions but rather in the presence of stressful stimuli, which revealed the greater vulnerability of motor neurons derived from M337V mutant iPSCs versus control iPSCs. In our paper (2), we addressed the fact that our results from the LDH release assay measuring cytotoxicity [fig. S11C in (2)] confirmed our results from the cell survival assay [fig. S1, A and B, in (2)]. In the cell survival assay, we transfected motor neurons with HB9::GFP lentivirus, and we counted total green fluorescent protein (GFP)–positive motor neurons once every other day, focusing on overall cell survival at a fixed time point (day 10). We concluded that there was no difference in the cell viability of motor neurons derived from mutant M337V iPSCs versus control iPSCs.

Bilican et al. point out in their Letter (1), and we agree, that estimating an increased risk of death by real-time single-cell longitudinal survival analysis (single-cell assay) is an elegant approach. They used this approach in their paper (3) and determined a difference in survival of mutant iPSC–derived versus control iPSC–derived motor neurons over a 10-day time period under basal condition [Fig. 5B in (3)]. In their study, they used an HB9::GFP plasmid vector to label motor neurons. Regarding this point, Bilican et al. note that real-time single-cell longitudinal survival analysis may be a more sensitive technique for detecting dynamic phenotypes that may otherwise be masked by variations arising from static measurements. Their new results using the LDH release assay presented in their Letter do not show differences in cytotoxicity under basal conditions [Fig. 1A in (1)]. Other sensitive assays for detection of cytotoxicity under basal conditions such as caspase-3 cleavage could also be used.

In our study (2), we used the HB9::GFP lentivirus vector, which integrated into the genomic DNA of motor neurons with stable expression of the GFP fluorescence signal. We observed GFP expression by motor neurons for 10 days after repassage of the cells infected by HB9::GFP lentivirus 2 weeks previously. We suggest that labeling by HB9::GFP lentivirus may make it possible to continue analyzing GFP–positive motor neurons in long-term cultures for more than 10 days and to determine whether death of motor neurons under basal conditions can be recapitulated. We agree with Bilican et al. that clonal variation can make it difficult to identify potentially important survival phenotypes. It may take different techniques to interpret subtle cellular vulnerabilities of motor neurons labeled with the HB9::GFP plasmid vector (3) versus the HB9::GFP lentivirus vector (2). Discussion concerning labeling methods for specific cell types derived from iPSCs is extremely important for the accurate assessment of cellular vulnerabilities.
We hope that Bilican et al.’s Letter and our Response will contribute to a clearer understanding of the subtle vulnerabilities of motor neurons derived from amyotrophic lateral sclerosis (ALS) patient iPSCs, which could become a useful platform for screening new drug candidates for treating this fatal disease.

REFERENCES

