Candidate ALS Therapeutics Motor toward "In Vitro Clinical Trials"

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Conducting drug discovery efforts in patient- and disease-specific cells can maximize their likelihood of success. In this issue of *Cell Stem Cell*, Yang et al. (2013) demonstrate the power of lineage-specific cell-based drug screens by identifying a compound that promotes survival of stem-cell-derived ALS mutant motor neurons.

Amyotrophic lateral sclerosis (ALS), more commonly known as Lou Gehrig's disease, is a notoriously intractable neurodegenerative disorder involving specific loss of motor neurons (MNs). As a result, patients suffer progressive paralysis and die due to loss of respiratory function (Maragakis, 2010; Robberecht and Philips, 2013). Causative mutations in superoxide dismutase-1 (SOD1) are found in 20% of inherited-ALS patients (Robberecht and Philips, 2013). Currently, the only FDA-approved drug for treatment of ALS is Riluzole, which has merely modest effects, extending patient lifespan by several months. Efforts to find more effective drugs have yielded two promising compounds, olesoxime and dexpramipexole, but the results of recent clinical trials were not promising (Cudkowicz et al., 2011; Robberecht and Philips, 2013).

One of the major obstacles in studying neurodegenerative diseases is the difficulty of obtaining relevant cell types for analysis. In the case of ALS, MNs are at the root of disease pathophysiology. Unfortunately, culturing MNs is very difficult, and it is almost impossible to biopsy sufficient numbers of MNs from patients for extensive study (Maragakis, 2010). Recent progress in stem cell biology, especially the development of induced pluripotent stem cell (iPSC) technology, provides immense opportunities for modeling human disease and screening potential therapeutics, using disease-relevant cell populations. For instance, after generating iPSCs from fibroblasts of ALS patients (Dimos et al., 2008), MNs from these ALS patient-specific iPSCs were used for validating potential candidate drugs and identifying their mechanisms of action (Egawa et al., 2012).

In this issue of *Cell Stem Cell*, Yang et al. (2013) provide a conceptually novel strategy to discover drugs for treatment of ALS. In their study, the authors differentiated mouse embryonic stem cells (ESCs) carrying either wild-type or mutant human *SOD1* and obtained large number of MNs. Because other studies found that trophic factor withdrawal causes significant death (around 80%) of MNs carrying wild-type or human mutant *SOD1* (Kieran et al., 2008), Rubin and colleagues developed an assay based on this paradigm and screened approximately 5,000 small molecules to identify compounds that prevent cell death of their mouse-ESC-derived MNs (Yang et al., 2013). The use of MNs bearing human wild-type or mutant SOD1 resulted in identification of 22 compounds that showed significant protective effects. Primary hit from this screen included compounds such as inhibitors of apoptosis, a matrix metalloprotease (MMP) inhibitor with agonist activity at cannabinoid receptors, and a calpain inhibitor. These compounds have been previously reported to have effects in ALS mice models, providing useful validation of their screen.

Among the remaining compounds, including several kinase inhibitors, the authors focused on Kenpaullone as a "hit" because it strongly increased survival of both wild-type and *SOD1* mutant MNs. Although Kenpaullone is a known GSK-3 inhibitor, its capacity to promote MN survival was significantly greater compared to other GSK-3 inhibitors tested. The authors then demonstrated that this is due to Kenpaullone's ability to inhibit HGK, which acts as an upstream regulator of a stress-induced neuronal cell death signal through a Tak1-MKK4-JNK-c-Jun pathway. Because CHIR99021 (another GSK-3 inhibitor tested here) could not rescue MN death in this experimental setting, HGK could be considered a new therapeutic target for further drug discovery. Whether inhibition of HGK alone is effective in preventing MN death, or whether it requires concurrent inhibition of GSK-3, requires additional investigation. In addition to Kenpaullone's effect on cell survival, it preserves morphology and electrophysiological activity even after long-term treatment, which suggests additional corrective benefits to MNs upon chronic treatment.

The authors then extended their validation of Kenpaullone to human MNs. They found that Kenpaullone promotes survival of human ESC (hESC)-derived MNs, as well as MNs harboring *SOD1* mutations from patient-specific iPSCs. More importantly, Kenpaullone can prevent death of MNs carrying mutations in *TDP-43*, another major genetic defect found in congenital ALS (Robberecht and Philips, 2013). Intriguingly, the authors also tested the effects of olesoxime and dexpramipexole. These compounds appeared promising in mouse studies but did not fare well in clinical trials (Cudkowicz et al., 2011; Robberecht and Philips, 2013). Compellingly, these compounds were not successful in rescuing death of MNs carrying human *SOD1* mutations. This finding is a powerful example of "preclinical testing in a dish." Such preliminary screening steps can potentially save a huge amount of resources and accelerate

drug discovery by excluding ineffective drugs before they proceed to clinical trials.

One fascinating effect of Kenpaullone is its ability to rescue cell death of MNs carrying mutations not only in *SOD1* but also in *TDP-43*. It remains to be seen whether Kenpaullone has similar effects on MNs harboring other ALS-specific mutations, such as *C9orf72* (Robberecht and Philips, 2013), or MNs from patients with sporadic ALS. If Kenpaullone can rescue MNs with distinct causative mutations, it may also act positively on other types of afflicted neurons in ALS patients. However, the mechanism behind the effects of Kenpaullone on MNs carrying distinct disease-inducing mutations needs to be clarified. It is possible that a compound with these pleiotropic effects was revealed due to the design of the primary screen, as the trophic factor withdrawal assay may not reflect a primary cause of MN death in ALS.

ESC- and iPSC-based screens such as the one performed by Yang et al. require relatively pure populations of the cell type of interest. Here, Yang et al. show MN differentiation efficacy of 30%∼50%, a significant improvement over previous efforts (Wichterle et al., 2002). They attempt to obtain MN cultures with even higher purity after primary screening by Ara-C treatment to kill mitotic cells and fluorescence-activated cell sorting (based on GFP expression of the *SOD1* transgene). Despite these efforts, the non-MN population in primary screening may mask true effects or cause artifacts, and we cannot exclude the possibility that any of the 22 primary hit compounds might act indirectly to promote neuronal survival. It is well known that deriving pure MN populations from iPSCs is challenging, and the issues described above emphasize the importance of developing more-efficient MN differentiation protocols for use in divergent applications.

As mentioned by the authors (Yang et al., 2013), the real value of stem-cell-based screening is the identification of relevant hit compounds suitable for moving into animal models of disease for proof of efficacy. Complex live-animal models provide further practical difficulties, such as stability of the compounds, toxicity, pharmacokinetic properties, and ability to penetrate the blood-brain barrier, which must be overcome for initial hits to show translational potential. Because other compounds from recent drug screening efforts using patient-specific hiPSCs (Choi et al., 2013; Lee et al., 2012) have not been tested in any in vivo studies, it will be interesting to see whether the hit compounds found from all of these stem-cell-based screening approaches exhibit positive results in animal models, as well as in clinical tests, in the near future.

In summary, Yang et al. (2013) showed the possibility of customized drug validation using patient-specific iPSCs. This is one of the best examples of personalized medicine, particularly from the perspective of drug discovery, for a disease with different genetic mutations. While this is the first trial to identify candidate ALS therapeutic agents using stem cells, which could result in discovering novel ALS-relevant drugs, the future is bright for targeted drug screening in a variety of diseases that require otherwise-limited cell populations.

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