Identification of New Hit Compounds Using a High-Throughput Phenotypic Screen with SMA Patient iPSC-Derived Motor Neurons

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Introduction

Spinal muscular atrophy (SMA) is an inheritable cause of infant mortality that is characterized by the loss of lower motor neurons and skeletal muscle weakness. The degeneration of motor neurons is caused by insufficient levels of survival motor neuron (SMN) protein, which is encoded by two nearly identical genes SMN1 and SMN2. Most cases of SMA harbor homozygous deletions of the SMN1 gene and retain at least one copy of SMN2. Hence, a promising treatment strategy is to upregulate the levels of the full-length SMN protein originating from the SMN2 gene. Drug discovery screening platforms typically use SMA fibroblasts or lymphocytes, yet the identified molecules often had limited efficacy in SMA mouse models, especially reconstituting motor neuron (MN) degeneration. Therefore, MNs from SMA patients should be used early in drug discovery to increase the likelihood of identifying effective small molecule therapeutics. At BrainXell, we have established new technologies to rapidly differentiate SMA patient induced pluripotent stem cells (iPSCs) into large quantities of neurons. We have used genome editing to endogenously fuse SMN2 with a nanoluciferase (NLuc) reporter, which enables high-throughput screening (HTS) that monitors the expression levels of SMN after 48 h exposure to each compound. The assay was adapted to meet HTS requirements, including: large batch sizes, 1536-well format, minimal well-to-well variation, short-term culture, plating by automated dispenser, and low reagent volumes. Applying a quantitative HTS approach, we screened the LOPAC, NPC, and MIPE libraries (~9,000 compounds) in a dose-responsive manner. After demonstrating feasibility, we expanded the screen to the larger Genesis library (~95,000 compounds) in order to identify novel hit molecules. Compounds that increased SMN2 expression by >20% were considered hits. Analysis of the combined ~105,000 compound qHTS identified 81 candidate compounds, which were re-screened in triplicate. Ten compounds increased SMN2 expression by at least 20% with EC50 < 10 µM. We then used an ELISA to validate the increased SMN2 expression after 48 h treatment. This screening paradigm identified and validated at least one new hit compound that has promising efficacy, but the potency will require optimization.

Materials and Methods

Establishment of Reporter iPSC Lines

Human iPSC lines, SMA232 were applied in this study. All iPSCs were cultured on irradiated mouse embryonic fibroblasts (MEFs) as described in the standard protocol (www.wicell.org). We applied CRISPR technology to integrate reporter NLuc into the survival motor neuron 2 (SMN2) locus. CRISPR guide RNA pairs, Cast-Nickase and Donor plasmid were introduced into iPSCs by electroporation. Neomycin was added in the culture medium to select the resistant cells. The neomycin-resistant iPSC colonies were picked and screened by PCR to detect the integration of the reporter plasmid. Neomycin-resistant iPSC lines were confirmed without mutation in SMN2 gene or off-target sites.

Neuron Differentiation from Human iPSCs

Motor Neuron differentiation from human iPSCs was based on protocols described previously (Du et al. 2015. Nat Commun. 6:6626). Briefly, human iPSCs were treated with small molecules for 1 week to induce neuroepithelial progenitors (NEPs). These progenitors were expanded with the combination of small molecules and frozen in cell freezing medium. To accelerate maturation after thawing and seeding, neurons were cultured in medium supplemented with BrainXell Seeding Supplement for another 1-2 weeks.

Multielectrode Array (MEA) Analysis

NEPs were split and treated in additional patterning molecules for another 1 week to generate sub-type specific neuron progenitors. These progenitors were expanded with the combination of small molecules and frozen in cell freezing medium. To accelerate maturation after thawing and seeding, neurons were cultured in medium supplemented with BrainXell Seeding Supplement for another 1-2 weeks.

Quantitative HTS Screening

MNPs were thawed and plated with a liquid handling system (MultiDrop) at 1,200-1,500 cells/well in 4 µL into all-well uncoated 1536-well plates (unless otherwise noted). On Day 1 (24 hours after thawing and plating), compounds were added by pin tool. On Day 7, the plate was detected using the Nano-Glo Luciferase Assay kit (Promega). Luminescence signal was measured with a Varioscan Lx system (PerkinElmer). Hits were confirmed using equivalent conditions in a 384-well format.

Letters corresponding to respective data point.

Table 1. Summary of qHTS from Each Library Screen.

<table>
<thead>
<tr>
<th>Library</th>
<th>Number of Compounds</th>
<th>CV</th>
<th>Response Window</th>
<th>Initial Hits</th>
<th>Verified Hits</th>
</tr>
</thead>
<tbody>
<tr>
<td>LOPAC</td>
<td>1,280</td>
<td>8%</td>
<td>25</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>NPC-A</td>
<td>1,408</td>
<td>8%</td>
<td>38</td>
<td>2</td>
<td>1</td>
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<tr>
<td>NPC-B</td>
<td>1,408</td>
<td>8%</td>
<td>38</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>MIPE</td>
<td>1,920</td>
<td>12%</td>
<td>36%</td>
<td>29</td>
<td>9</td>
</tr>
<tr>
<td>Genesis Library</td>
<td>95,000</td>
<td>42%</td>
<td>63% (DMSO)</td>
<td>29</td>
<td>9</td>
</tr>
</tbody>
</table>

References