High-Throughput Screening of Small Molecules in PLP-EGFP Schwann Cells to Identify Modulators of Myelination

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Myelin sheaths are often the primary site of damage during the initial stages of pathological insults, including immune assaults or genetic defects seen in demyelinating neuropathies such as Charcot-Marie-Tooth disease, PNS tumors or metabolic disturbances, leading to severe neurological deficits. Schwann cells are essential for peripheral nerve homeostasis, axonal support and myelination formation. Proteolipid protein 1 (PLP1) is a myelin protein expressed in Schwann cells and associated with the maintenance of myelination. The identification of drugs able of restoring Schwann cell function and myelination remains challenging.

A transgenic mouse model, PLP-EGFP C57BL/6J (RRID:IMSR JAX:033357) in which the GFP gene is expressed under the promoter of the PLP1 gene, was employed to purify and expand Schwann cells by adapting the protocol described by Kim and Kim (2018; DOI: 10.1007/978-1-4939-7649-2_3). Spindle-shaped Schwann cells were seeded (5000 cell/well) in 384-well plates and incubated for 24h at 37°C 5% CO_2. A set of 1120 compounds, marked drugs and pharmacologically active agents, from Prestwick library were evaluated in a single point assay at 10 µM with 0.1% DMSO. Brightfield and fluorescence images were acquired in a Operetta CLS High-Content Analysis System and fluorescence intensity was determined before and after different times of compound addition (0, 24, 48 and 72h) with the Harmony analysis software. This assay allowed the identification of six compounds that increased PLP1-GFP expression, which were subsequently confirmed in an independent assay.

Therefore, a primary Schwann cell culture was developed as a physiologically relevant model for performing high-throughput screening, providing a valuable platform to identify compounds that enhance myelination and may be relevant to the treatment of peripheral neuropathies.

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