Evaluate neural stem cells for neurodegenerative disease models

Neural stem cell–specific protein expression and mitochondrial assessment.

Parkinson's disease (PD) is a progressive neurodegenerative disorder that affects 1% of people over age 60 and more than 5 million people worldwide [1]. PD results from the selective loss of dopaminergic neurons in the substantia nigra region of the brain. The loss of these neurons initially affects movement. As the disease progresses, however, cognitive function is impaired, and late-stage disease is often accompanied by dementia. The absence of physiologically relevant cellular models for PD represents a major bottleneck for PD research. The development of suitable PD models would not only accelerate the discovery of disease mechanisms and drug targets but also serve a critical role in screening for clinical and therapeutic strategies.

Patient-specific iPSCs (induced pluripotent stem cells) have become attractive tools for disease modeling *in vitro*. Our research collaboration with The Parkinson's Institute (Sunnyvale, California) has set about to develop PD model systems using neural stem cells (NSCs) derived from iPSCs that were generated from diseased fibroblasts collected at the institute. NSCs are self-renewing multipotent progenitors that can be differentiated to become neurons. Here we describe two assays for assessing NSC identity and health: the Human Neural Stem Cell Immunocytochemistry Kit, which provides reagents and a protocol for convenient image-based analysis of four common markers of human NSCs, and the MitoSOX® Red Mitochondrial Superoxide Indicator, which provides a straightforward method for measuring mitochondrial superoxide levels in cells. These assays were employed in the evaluation of NSCs generated as one step in the development of neurodegenerative disease cell models targeted for PD research.

Detecting NSC-specific protein expression

The first step in evaluating NSCs as a PD disease model is to confirm their cell type. The expression of four well-established protein markers (nestin, Sox1, Sox2, and Pax6) is a fundamental indicator

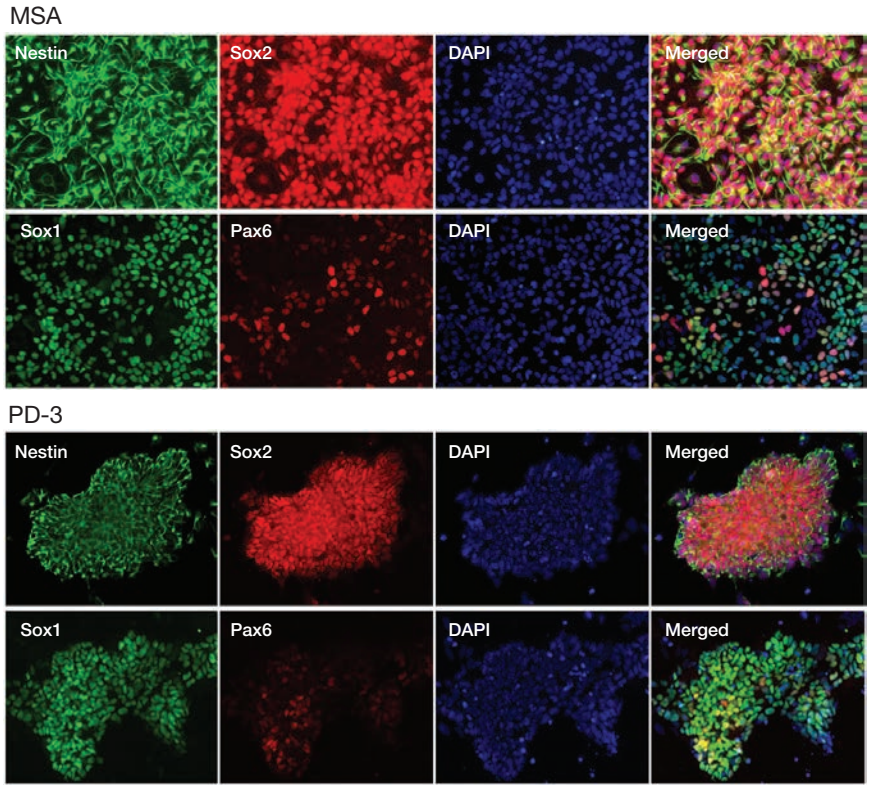

Figure 1. Immunocytochemical detection of NSC-specific markers on NSCs generated from MSA- and PD-affected donors. NSCs (passage 3) were cultured in Neural Expansion Medium (50% Neurobasal® Medium, 50% Advanced™ DMEM/F-12, and 1X Gibco® Neural Induction Supplement) on Geltrex® matrix–coated chamber slides for 2 days. After a 15 min fixative step, cells were permeabilized for 15 min, blocked for 1 hr, and then incubated with the antibodies for nestin (green), Sox2 (red), Sox1 (green), and Pax6 (red) and counterstained with DAPI nucleic acid stain (blue); all reagents are provided in the Human Neural Stem Cell Immunocytochemistry Kit (Cat. No. A24354). Images were captured using the EVOS® FLoid® Cell Imaging Station. Six NSC lines stained positive for the markers nestin, Sox2, Sox1, and some level of Pax6; representative images from the MSA NSCs and the PD-3 NSCs are shown here.

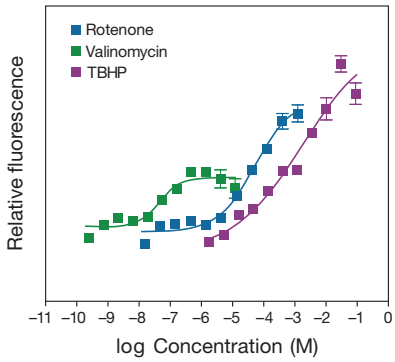

Figure 2. Detection of oxidative stress in PD-3 NSCs using MitoSOX® Red Mitochondrial Superoxide Indicator. PD-3 NSCs were cultured in StemPro® NSC SFM (Cat. No. A1050901) for 24 hr at 37°C in 384-well assay plates coated with CTS™ CELLstart™ substrate (Cat. No. A1014201). After a 1 hr incubation with 5 µM MitoSOX® Red Mitochondrial Superoxide Indicator (Cat. No. M36008), the cells were washed once with growth medium and treated with the stressor compounds for 2 hr, and the resultant fluorescence was measured on a Tecan® Safire²™ Fluorescence Plate Reader. The PD-3 NSCs showed the expected increase in oxidative stress with the increase in the concentration of rotenone, valinomycin, or *tert*-butyl hydroperoxide (TBHP).

of the identity and quality of a NSC. We evaluated six different patient-derived cell lines for NSC-specific protein expression. Disease-specific fibroblasts from three PD-affected donors (PD-1, PD-2, and PD-3), one donor affected by multiple systems atrophy (MSA), and two age-matched, healthy control individuals were reprogrammed into iPSC lines. The fibroblasts from the three PD donors had previously been genotyped and were known to contain common PD-related mutations in the genes *LRRK2*, *GBA*, and *PARK2*, whereas the MSA cell line was from a sporadic case with no mutations in genes known to be associated with PD. MSA has been described as a more severe form of PD, characterized by faster disease progression after the first appearance of symptoms [2].

After the six iPSC lines were analyzed for successful reprogramming, they were differentiated into NSCs using Gibco® PSC Neural Induction Medium. PSC Neural Induction Medium provides a means of generating NSCs from iPSCs in only 7 days, without the need for embryoid body (EB) formation [3]. Using the Human Neural Stem Cell Immunocytochemistry Kit, we demonstrated that all six NSC lines expressed the known neural protein markers nestin, Sox1, Sox2, and Pax6 (Figure 1). The Human NSC Immunocytochemistry Kit includes an optimized set of primary and secondary antibodies, the blue-fluorescent nucleic acid stain DAPI, and all of the buffers necessary to complete the staining protocol.

Monitoring mitochondrial activity as an indicator of cell health

NSC health is an important parameter to monitor when assessing the cellular effects of drugs, environmental factors, and biological modifiers. Because cell health cannot be easily defined using a single physiological attribute, it is often desirable to use several different cell function indices. To this end, the derived NSCs were expanded and tested with a panel of assays that are particularly useful for indicating neural cell health, as each measures a different aspect of cell vitality.

One of the cell function probes used was the MitoSOX® Red Mitochondrial Superoxide Indicator, which exhibits increased red fluorescence with increased mitochondrial superoxide levels and can easily be detected on a fluorescence plate reader. NSCs are characterized by high metabolic activity that is dependent on robust mitochondrial function. To assess their mitochondrial activity, we treated the NSCs with three different cell stressors—rotenone, valinomycin, and *tert*-butyl hydroperoxide (TBHP)—in order to inhibit the electron transport chain, disrupt the transmembrane concentration gradient, and induce oxidative stress, respectively. All six of the NSC lines developed in this research collaboration exhibited the expected mitochondrial reactions in response to these cellular stressors (Figure 2).

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Although these assays were only two of the many analyses performed to assess the NSCs, this work has set the stage for a "disease in a dish" PD model using fibroblasts from PD-affected donors. To see more of the data produced through our collaboration with The Parkinson's Institute, read our three white papers accessible at **lifetechnologies.com/parkinsonsbp70**. There you can also learn about more products and protocols in our stem cell research portfolio. ■

References

1. Olanow CW, Stern MB, Sethi K (2009) *Neurology* 72:S1–S136.
2. Burn DJ, Jaros E (2001) *Mol Pathol* 54:419–426.
3. Yan Y, Shin S, Jha BS et al. (2013) *Stem Cells Transl Med* 2:862–870.

Product	Quantity	Cat. No.
CTS™ CELLstart™ Substrate	2 mL	A1014201
Human Neural Stem Cell Immunocytochemistry Kit	20 tests	A24354
MitoSOX™ Red Mitochondrial Superoxide Indicator, for live-cell imaging	10 x 50 µg	M36008
PSC Neural Induction Medium	500 mL	A1647801
StemPro® NSC SFM (Neural Stem Cell Serum-Free Medium)	1 kit	A1050901