

Identification of stimulators and inhibitors of cell migration in human embryonic stem cell derived neural progenitors using a novel, high throughput amenable assay platform

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Abstract

Migration of neural progenitors is an important process for the proper development and maintenance of the nervous system. Neural progenitors are created in proliferative zones within the brain and migrate to specific destinations guided by various extracellular cues, including various neurotrophic factors (Marin and Rubenstein, 2003; Marin et al., 2003). In addition, some developmental neurotoxicants have been shown to interfere with the migratory behavior of neural stem cells (e.g., Moors, et al., 2009).

We investigated the migratory behavior of an adherent monolayer neural progenitor cell line derived from human embryonic stem cells (hNP1TM; Aruna Biomedical) using a novel 96-well based cell migration assay platform (OrisTM Cell Migration Assay; Platypus Technologies) amenable for high throughput screening. The assay platform uses stoppers to create central exclusion zones within the wells; cells are plated outside the zone and migrate inward once the stopper is removed.

Using the OrisTM assay, we demonstrated that neurotrophic factors used to promote proliferation and differentiation also have chemokinetic effects on neural progenitors. We further demonstrated that a known inhibitor of cell migration, Cytochalasin D, can block the ability of neural progenitors to migrate.

Together, these data demonstrate an assay system that can be used to readily identify factors that promote or inhibit neural progenitor cell migration. The combination of this novel cell migration assay and neural stem cells will provide a powerful tool for understanding proper nervous system development, development of therapies for cell migration defects, and identifying novel environmental neurotoxicants.

Methods

- hNP1TM cells were grown as adherent monolayers using Aruna's defined basal medium plus supplement (AB2 + ANS) with recombinant bFGF (basic fibroblast growth factor) and LIF (leukemia inhibitory factor) included on surfaces coated with Matrigel (1:200).
- hNP1TM were plated at 60,000 cells per well onto Matrigel-coated OrisTM Assay plates in proliferation medium (PRO) for ~16 hrs at 37°C.
- Stoppers were then removed, except in the "no migration" control column (STOPPER), and the plating medium was replaced with test medium (e.g., differentiation medium aka DIF).
- Cells were incubated at 37°C for 72 hrs, stoppers removed from "no migration" control wells and all cells were stained at 37°C for 30-60 minutes with calcein (5 µg/mL) in phenol red-free Neurobasal medium with 1% BSA.
- Plates were read using a Flexstation3 microplate reader (ex 494 nm/ em 517 nm) and then imaged by epifluorescence microscopy.

References

- Marin O, Rubenstein JL. (2003) Cell migration in the forebrain. *Annu Rev Neurosci.* 26:441-83.
- Marin O, et al. (2003) Directional guidance of interneuron migration to the cerebral cortex relies on subcortical Slit1/2-independent repulsion and cortical attraction. *Development.* 130(9):1889-901.
- Moors M, et al. (2009) Human neurospheres as three-dimensional cellular systems for developmental neurotoxicity testing. *Environ Health Perspect.* 117(7):1131-8.
- Shin S, et al. (2006) Long-term proliferation of human embryonic stem cell-derived neuroepithelial cells using defined adherent culture conditions. *Stem Cells.* 24(1):125-38.
- Young A, et al. (2010) Glial cell line-derived neurotrophic factor enhances in vitro differentiation of mid-/hindbrain neural progenitor cells to dopaminergic-like neurons. *J Neurosci Res.* 88(15):3222-32.
- Zhang JH, et al. (1999) A Simple Statistical Parameter for Use in Evaluation and Validation of High Throughput Screening Assays. *J Biomol Screen.* 4(2):67-73.

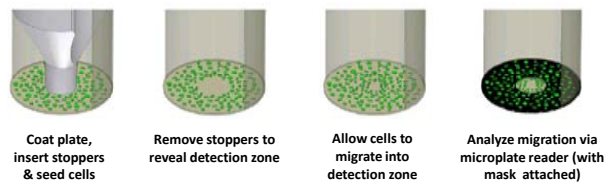


Figure 1. Schematic of the OrisTM Cell Migration Assay

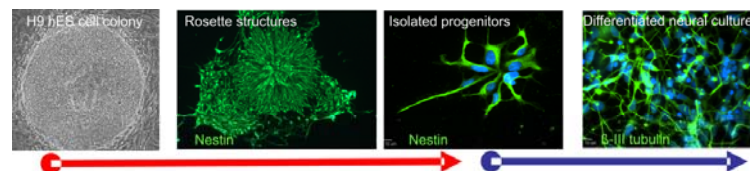


Figure 2. Derivation of hNP1TM neural progenitors. hNP1TM cells were originally derived from the H9 (WA09) human embryonic stem cell line using defined, serum-free conditions (e.g., Shin, et al., 2006), proliferate as adherent monolayers and maintain a stable karyotype for multiple (>10) passages, making them scalable for HTS format (96-, 384-well) assays. hNP1TM express proneural markers (>90% nestin+ and <5% Oct4+) and are capable of differentiation into multiple neuronal phenotypes (e.g., dopaminergic neurons; Young, et al., 2010) upon withdrawal of bFGF.

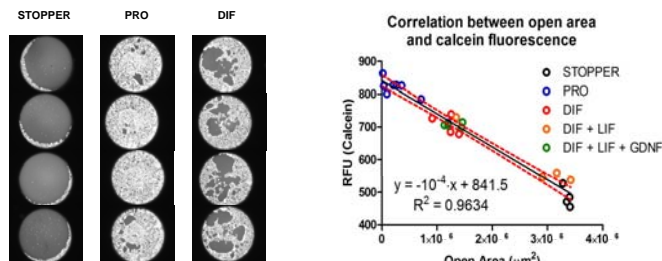


Figure 3. Use of calcein to measure hNP1TM cell migration on a microplate reader. To develop a homogenous HTS-suitable assay, we tested the cytoplasmic dye calcein as a surrogate for time- and computationally-intensive area measurements of detection zone coverage by hNP1TM cells. Cells were incubated for 72 hrs in proliferation (PRO) or differentiation (DIF) media with and without LIF and GDNF. Calcein fluorescence correlated well with area measurements obtained using ImageJ. Subsequent migration experiments were therefore analyzed for fluorescence signal using the microplate reader as a rapid means to capture data.

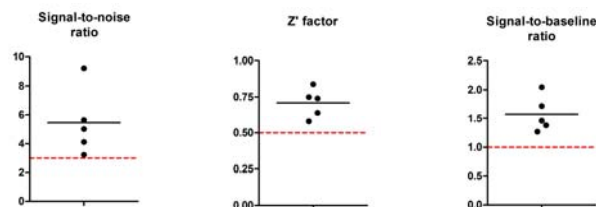


Figure 4. Assay performance measures for the hNP1TM OrisTM Cell Migration Assay. To assess assay performance, we calculated the signal-to-noise ratio, signal-to-baseline ratio, and the Z' factor (Zhang, et al., 1999) for 5 independent experiments using hNP1TM cells migrating in PRO medium. All 3 parameters were in the range considered acceptable for HTS-suitable assays.

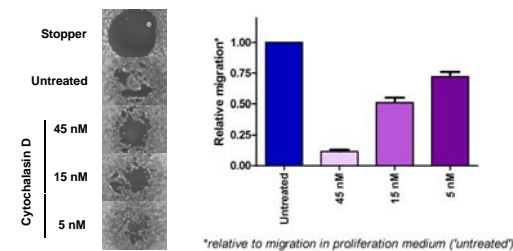


Figure 5. Inhibition of hNP1TM cell migration by Cytochalasin D. Cytochalasin D inhibited hNP1TM migration in a concentration dependent manner with an IC₅₀ of ~15 nM (72 hr treatment; the mean of 2 independent experiments shown here), indicating that inhibitors of migration can be readily detected.

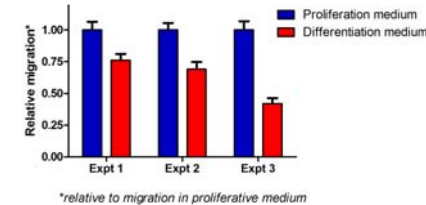


Figure 6. Differences in hNP1TM cell migration under various culture conditions. hNP1TM cells showed reduced migration in differentiation medium (basal medium alone) than in proliferation medium (basal medium plus bFGF and LIF), suggesting that either bFGF, LIF or both have a chemokinetic effect on neural progenitors.

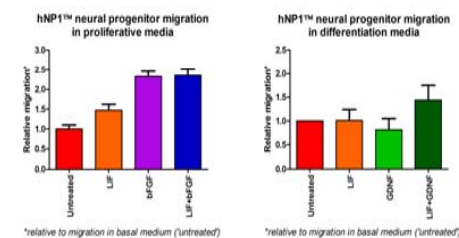


Figure 7. Effect of growth factor combinations on hNP1TM cell migration. Preliminary results indicate that bFGF alone is primarily responsible for driving migratory behavior under proliferative conditions. In contrast, the combination of LIF and GDNF, which differentiates hNP1TM into dopaminergic neurons (Young, et al., 2010), has a synergistic chemokinetic effect.

Conclusions

- The hNP1TM OrisTM Cell Migration Assay can quantitatively detect both stimulators and inhibitors of cell migration.
- Method development to date indicates that the assay has the potential for adaptation as a homogenous HTS-suitable cell-based assay.
- Preliminary results suggest that bFGF alone has a potent chemokinetic effect while LIF and GDNF act synergistically to drive migratory behavior during dopaminergic differentiation.

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