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Application Note

ReNcell® Human Neural Progenitors: Renewable and Consistent Supply of Human Functional Neurons

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Abstract

ReNcell® VM and ReNcell® CX are two well-established neural stem cell lines derived from developing human brains. ReNcell® VM and CX cells are generated from the ventral mesencephalon and cortical regions of the brain, respectively, and transduced with the myc transcription factor. Both cell lines offer phenotype and genotype stability, in addition to the multipotential neuronal differentiation capacity, over long-term culture. This article describes the characteristics and differentiation of ReNcell® lines. Results of calcium and membrane potential changes in response to various ligands are also shown. The convenience to maintain in culture and flexibility to differentiate to individual scientists' needs make ReNcell® lines the ideal platform for research and discovery.

Introduction

Neural stem cells (NSCs) were first described in the rodent brain by Reynolds and Weis in 1992[1]. Seven years later, the isolation of human NSCs was documented by Vescovi et al.[2] Since those initial findings, NSCs have been valuable tools for neuroscience, signal transduction and developmental biology, to name but a few.

Standardized platform

Early studies using human NSCs were limited by the short-term stability of genotype and phenotype in culture. However, immortalization of human NSCs with the myc transcription factor has proven highly effective at overcoming these challenges. ReNcell® VM and CX lines were immortalized using myc technology. Myc is believed to drive and sustain self-renewal and proliferation of the stem cell, thus keeping differentiation at bay until desired. An up-regulation of telomerase activity is observed in myc transduced ReNcell®, which lends itself to a stable genotype in culture. Traditionally thought of as a proto-oncogene, it now appears that myc may be a "stemness" gene. An exciting discovery was made when Takahashi and Yamanaka demonstrated that a fibroblast cell could be transformed into a stem cell by using only four genes: c-Myc, Oct4, Klf4, and Sox2[3]. This finding has since been corroborated by independent research groups.

ReNcell® lines are regularly tested to ensure that they are free from any adventitious agents or contaminants (e.g. mycoplasma), thus guaranteeing their safety. ReNcell® VM and CX cell lines are easily maintained as monolayer cultures in serum-free ReNcell® maintenance medium (Cat. No. SCM005) using laminin coated T-culture flasks. Passaging the ReNcell® cell line is carried out every 3-4 days with typical yields of approximately 4 x 10^6 cells per T75 cm^2 flask. Both cell lines can be maintained long-term in culture and readily cryopreserved without any adverse effects on genotype or phenotype. The consistent and renewable supply of NSCs generated by ReNcell® VM and CX cell lines make them an excellent tool in stem cell research.

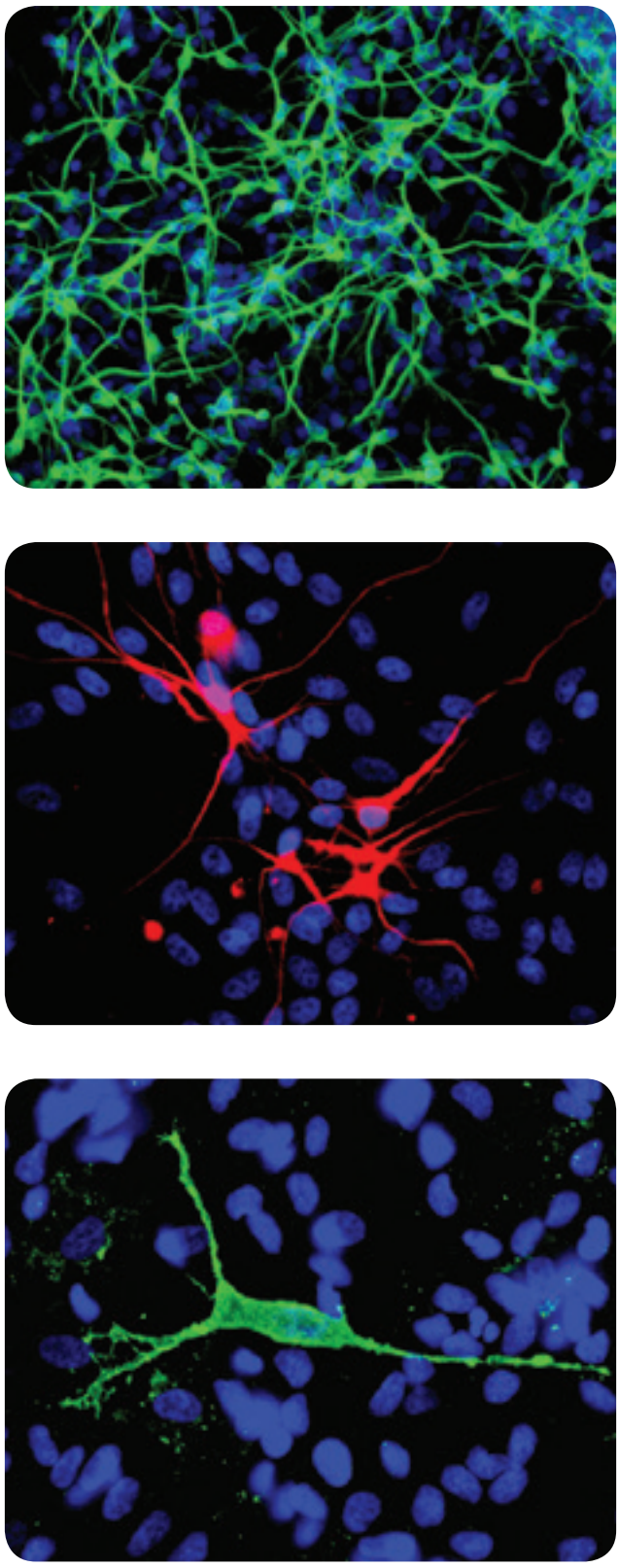

Figure 1. Multipotentiality of ReNcell®: Both cell lines readily differentiate into all three neuronal phenoytpes: neurons (bIII-tubulin, green, 20X, A); astrocytes (GFAP, red, 40X, B) and oligodendrocytes (Gal C, green, 60X, C); all counterstained with Hoechst nuclear stain (blue).

Differentiation as easy as 1, 2, 3

The advantages of myc transduction on cell growth and genotype have been described. However, it is important to note that myc transduction does not interfere with differentiation. ReNcell® VM and CX cell lines retain the ability to differentiate into all three neuronal subtypes - neurons, astrocytes, and oligodendrocytes. Indeed, the proteomics[4] and electrophysiology[5] of ReNcell® cell lines have been reported in the undifferentiated and differentiated state. These data indicate that both ReNcell® lines are true neural stem cells by their ability to expand in culture and their multipotent capacity to generate all three neuronal subtypes.

Differentiation of the cells occurs when growth is arrested by removal of growth factors bFGF and EGF from the tissue culture medium. ReNcells are compatible with all sizes of monolayer tissue culture plasticware for differentiation. We typically use 96-well plates because both cell lines perform well in this format and it allows high-throughput experimentation. Differentiation of both ReNcell® lines can be carried out in three steps. In the first step the cells are passaged as normal and plated at 10,000 cells per well into a laminin coated 96-well plate. ReNcell® Maintenance Medium containing growth factors EGF and bFGF is used at 100-200 µL per well and the cells are grown to confluency over 2-3 days. The second step involves inducing differentiation by replacing the medium with ReNcell® maintenance medium without EGF and bFGF. In as little as three days after induction, the cells go from a cobblestone stem cell morphology to cells containing extensive processes and mature neuronal morphologies. Analysis comprises the final step of the differentiation process. Immunocytochemistry, Western blot, and PCR are some of the most straightforward methods to demonstrate successful differentiation.

Flexibility and discovery

The standard three-step differentiation method described above provides a framework to tailor the experiment to novel research applications. Each stage can be modified depending on the researcher's needs and imagination. For example, prior to seeding cells in step 1 of the assay, the ReNcell® cell line can be seeded at the same density on uncoated plasticware in growth factor containing medium to form neurospheres over 5-7 days. The neurospheres can then be transferred onto laminin coated tissue cultureware in medium containing growth factors and the three differentiation steps carried out. The formation of neurospheres prior to initiation of the differentiation protocol has been shown to enhance electrophysiologically active neural differentiation in ReNcell® VM[5] cell line. As a further modification to step 1 in the differentiation protocol, the cells can be "pre-treated" with agents of interest during the growth phase. These agents may include development or regional specificity signals that prime the cells before differentiation. Growth factor withdrawal in step 2 induces differentiation in its simplest form; however,

the addition of agents that stimulate differentiation are ideally added at this stage. In our laboratory, we have found that the addition of 1mM dibutyrl-cAMP to the differentiation medium significantly enhances the dopaminergic differentiation of the ReNcell® VM cell line. As a final modification to the protocol, investigators may wish to incorporate survival factors following the induction of differentiation (e.g. GDNF at 2 ng/mL). Due to the flexibility of these cells, testing your compound of interest in ReNcell® VM and CX cell lines couldn't be easier.

Pharmacology of ReNcell® lines

We used a range of ligands to monitor the cytosolic calcium and membrane potential changes in an application of ReNcell® cell lines. Cells were grown in 96-well plates to generate undifferentiated or differentiated cells, and functional responses to agonists were monitored using a Flex Station® Fluorometer (Molecular Devices, Reading, UK). Both ReNcell® VM and CX cell lines responded to a range of stimuli including carbachol, ATP, and histamine (weakly). ReNcell® CX also showed a robust response to thrombin while 5-HT was inactive. Depolarisation of ReNcell® VM and CX membrane potential was seen with KCL. To date, only small hyperpolarization or depolarization responses to receptor ligands have been observed in ReNcell® cell lines, which suggests ion channel activity in a small sub-population of cells. In summary, ReNcell® VM and CX cell lines are ideal tools for discovery. Optimize your research output by working with human cell lines. ReNcell® maintenance and freezing media, along with our full technical support, guarantee you the best results.

References

1. Reynolds BA, Weiss S: Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. Science 1992, 255(5052):1707-1710.
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4. Hoffrogge R, Mikkat S, et al. 2-DE proteome analysis of a proliferating and differentiating human neuronal stem cell line (ReNcell® VM). Proteomics 2006, 6(6):1833-1847.
5. Donato R, Miljan EA, et al. Differential development of neuronal physiological responsiveness in two human neural stem cell lines. BMC Neurosci 2007, 8:36.

Related Products

Description	Cat. No.
ReNcell® CX Human Neural Progenitor Cell Line	**SCC007**
ReNcell® VM Human Neural Progenitor Cell Line	**SCC008**
ReNcell® CX Kit (ReNcell® CX cells and ReNcell® Maintenance Medium)	**SCC009**
ReNcell® VM Kit (ReNcell® VM cells and ReNcell® Maintenance Medium)	**SCC010**
ReNcell® NSC Maintenance Media	**SCM005**
ReNcell® Neural Stem Cell Freezing Medium	**SCM007**
Mouse Laminin Protein	**L2020**
Poly-L-Ornithine Solution	**P4957**
ECM Gel Matrix, Basement Membrane Extract	**L1270**
Anti-Nestin Antibody, clone 10C2	**MAB5326**
Anti-Sox2 Antibody Anti-SOX2 Antibody	**AB5603**
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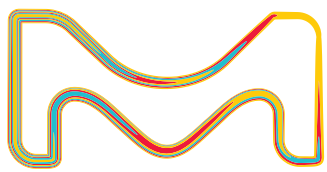

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