TECH NOTE

A fully defined, serum-free culture system for neural stem cell maintenance and differentiation

RHB-A cell culture medium

Improved maintenance and expansion of human neural stem cells >>

Differentiation of human pluripotent stem cells into neurons and astrocytes >>

Introduction

Differentiation into cells of neural lineage is a challenging and time-consuming process. Conventional differentiation media initially provided a baseline culture system for generating neural cells. However, these media were relatively inefficient, giving low percentages of neural cells, and cumbersome, in that the type of medium used for differentiation had to be changed completely before expansion of intermediate neural stem (NS) cells. Furthermore, these media were often specific only to mouse cells, necessitating the development of a medium compatible with human cells.

RHB-A medium is the next-generation formulation of N2B27-based medium for NS cell culture and neural differentiation. This optimized medium was developed to both improve the differentiation of human pluripotent stem cells into neurons, while also enabling the maintenance and expansion of NS cells upon supplementation with epidermal growth factor (EGF) and fibroblast growth factor-basic (FGF-basic).

In the following experiments, RHB-A medium was directly compared to different vendors' media for the expansion of human NS cells and was further tested for the differentiation of those expanded cells into both neurons and astrocytes.

Results

Maintenance of linear growth

A human NS cell line (Human Neural Cortex Stem Cell Line Kit, Y40050) was grown in either the RHB-A system or a system from one of two different vendors (Vendors A and B) for 35 days. The cells in the RHB-A system showed a linear growth curve until Day 28, whereas cells in Vendor A's system showed little growth during cultivation, and Vendor B's system was unable to maintain the NS cell culture.

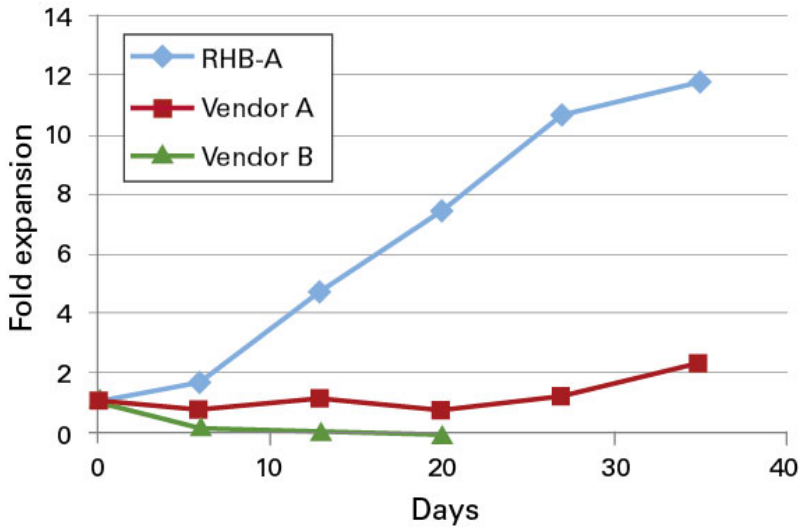

Figure 1. Fold-expansion profiles of human NS cell lines cultured in different media.

Differentiation into neurons and astrocytes

NS cells cultured for 28 days in the RHB-A system (as shown above) were then differentiated into neurons or astrocytes, as described in the Methods section. Following differentiation, cells were immunolabeled for microtubule-associated protein 2 (MAP2) and glial fibrillary acidic protein (GFAP), markers for neurons and astrocytes, respectively. Under conditions for neuron differentiation, most of the cells were positively labeled with an anti-MAP2 antibody but showed no labeling with the anti-GFAP antibody. On the other hand, under conditions for astrocyte differentiation, the culture showed mostly GFAP-positive cells and much lower number of cells showed positive staining for MAP2. Together, these results provide visual confirmation of the differentiation of NS cells into neurons and astrocytes while cultured in RHB-A medium.

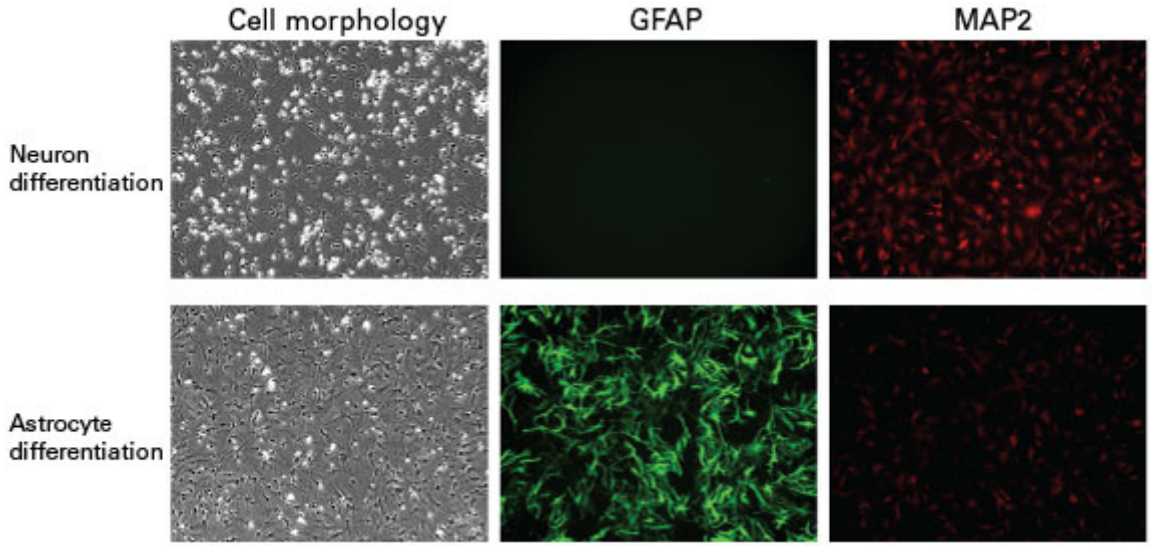

Figure 2. Immunolabeling of neuron marker, MAP2, and astrocyte marker, GFAP.

Conclusions

The RHB-A system enables stable proliferation of NS cell cultures, while maintaining neuronal and astrocytic differentiation potential. Thus, this second-generation N2B27 medium can be widely used as a basal medium for the

maintenance, expansion, and differentiation of NS cells. Flexibility is offered in the form of RHB-BASAL medium, which does not contain any growth factors or neuronal supplements, allowing it to be tailored to specific requirements depending on cell type.

Methods

Cell culture

The human neural cortex stem cell line (Human Neural Cortex Stem Cell Line Kit) was cultured for four weeks in the RHB-A system, StemPro NSC SFM (Thermo Fisher Scientific), or Stemline Neural Stem Cell Expansion Medium (Sigma-Aldrich Corp.). The cells were cultured according to each manufacturer's recommendations. Tissue culture plates precoated with 10 µg/ml of laminin (Thermo Fisher Scientific) were used for the RHB-A system, with EGF (PeproTech) and FGF-basic (PeproTech) at final concentrations of 20 ng/ml each in the medium. The cells were seeded at 4 x 10^4 cells/cm^2 and passaged once a week.

Differentiation into neurons or astrocytes

Human neural cortex system cells were cultured for four weeks in the RHB-A system. The resulting expanded cells were differentiated into neurons or astrocytes with the procedures described below.

For differentiation into neurons, cells were seeded at 25,000 cells/cm^2 on plates precoated with poly-L-ornithine and laminin, and cultured in RHB-BASAL medium supplemented with 0.5% NDiff N2-AF, 1% B27 supplement (Thermo Fisher Scientific), and 10 ng/ml of FGF-basic (Day 0). On Day 7, the differentiation medium was switched to a 1:1 ratio of RHB-BASAL medium to Neurobasal medium, minus phenol red (Thermo Fisher Scientific), supplemented with 0.25% NDiff N2-AF, 1% B27 supplement, 10 ng/ml of FGF-basic, and 0.5% GlutaMAX (Thermo Fisher Scientific). On Day 14, the differentiation medium was switched to Neurobasal medium, minus phenol red, supplemented with 2% B27 supplement, 20 ng/ml BDNF (PeproTech), and 1% GlutaMAX. The cells were cultured for an additional 14 days. During cultivation, the differentiation medium was changed every other day.

For differentiation into astrocytes, the cells were seeded at 25,000 cells/cm^2 on plates precoated with poly-L-ornithine and laminin, and cultured in RHB-A medium supplemented with 10 ng/ml of BMP-4 (R&D Systems) for 22 days. During cultivation, the differentiation medium was changed every other day.

After differentiation, GFAP and MAP2 expression levels were examined by immunolabeling.

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http://www.clontech.com/US/Products/Stem_Cell_Research/Resources/Technical_Notes/RHB-A_Neural_Stem_Cell_Medium