ReNcell™ VM Cell Line - A Model of Human Neural Development and Differentiation

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Abstract

ReNcell VM is a human neural stem cell line derived from the ventral mesencephalic region of the developing human brain and immortalized by retroviral transduction with the myc oncogene. This cell line offers a stable phenotype and genotype, in addition to its capacity to differentiate into several types of neuronal cells. Thus, ReNcell VM is an ideal, standardized, *in vitro*, human-based platform for drug discovery and research applications. This article demonstrates one such application wherein the proteomic changes were monitored during differentiation. A protein map identified changes in the expression levels of 146 distinct proteins associated with the terminal differentiation of these proliferating stem cells. A number of specific signalling pathways involved in the differentiation of human neural stem cells were recognized.

Introduction

There has been a great deal of excitement surrounding neural stem cells (NSCs) since their initial discovery in 1992. NSCs offer opportunities for the development of new research strategies because they provide an inexhaustible supply of neuronal phenotypes that previously could only be obtained from fresh primary tissue preparations. However, the capacity to maintain a stable phenotype and karyotype across multiple passages was limited with human NSCs. Furthermore, preparation of human NSCs from different donor tissues introduces additional variability to research studies. Immortalization of human NSCs with the myc transcription factor has proven highly effective at overcoming these limitations.

ReNcell VM line is characterized as a NSC because of its self-renewal capacity and multipotentiality following functional differentiation[1]. Due to its myc immortalization transduction, the ReNcell VM line can be grown as a monolayer culture on laminin in serum free medium without losing biological potency or developing karyotypic abnormalities. Myc is believed to drive and sustain self-renewal and proliferation of the stem cell, thus keeping differentiation and the proteomic changes associated with differentiation at bay until desired.

Methods

Differentiation of ReNcell VM cells was easily initiated by removing the growth factors, bFGF and EGF, from the tissue culture medium[2]. This resulted in growth arrest of the cells; within 2-3 days, they began to differentiate into a more neuronal and glial cell morphology that was visible by phase contrast microscopy. Crude protein extracts from proliferating (day 0) and differentiated ReNcell cells (4 or 7 days) were separated utilising high resolution 2-D gel separation. Fluctuation in individual protein spots was mapped by combining the gel images into a fusion gel (Delta2D software, Decodon, Greifswald, Germany). Identification of protein spots was carried out by double or multiple MALDI-TOF-MS analysis.

Results & Discussion

Upon the removal of growth factors, ReNcell VM cells clearly differentiated within 4 days as shown by the transition from an undifferentiated cobblestone morphology to more elaborate, mature processes (Figure 1)[2]. The morphological changes were linked to a significant alteration in the protein expression profile of the cells. Interestingly, only minor differences were seen between 4 and 7 day differentiation samples. This suggests that terminal differentiation of the human NSC line, ReNcell VM cells, was achieved only 4 days after induction, in contrast to much longer differentiation times generally reported for rodent-derived NSCs. From the 956 individual spots mapped, 146 protein spots displayed differences in their intensities that could be directly associated with differentiation. The number of up-regulated proteins was approximately equal to those that were down-regulated (77 were down regulated, while 69 were up-regulated).

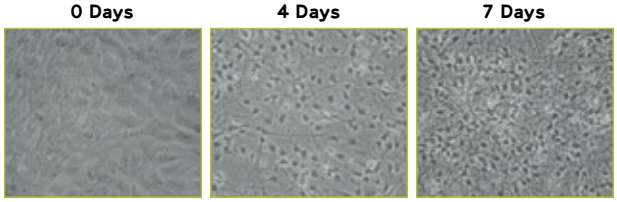

Figure 1. ReNcell VM cells at different stages of differentiation. ReNcell VM is a NSC line, which can be cultured as a monolayer (day 0) over multiple passages. Differentiation is initiated with the removal of growth factor from the culture medium. In as little as four days after induction the cells go from traditional 'cobblestone' stem cell morphology to cells containing extensive processes and mature neuronal morphologies.

The proteins that changed during differentiation were classified in to 21 functional categories based on information from the Gene Ontology database (**www.expasy.org/sprot**). The number of proteins in each functional group that changed upon differentiation was expressed as a percentage (Figure 2)[2]. The most significant changes in protein expression were related to protein synthesis, metabolism, processing, and degradation (21.3%). This was followed by changes in functional groups related to: cytoskeletal proteins (10.7%), stress response proteins (9.1%), RNA/other nucleic acids metabolism and transport, nuclear proteins (9.1%), signal transduction proteins (8.5%), and many others to a much lesser extent. Validation of these results was carried out by quantitative Western blot analysis of certain proteins found to be up-regulated and down-regulated by differentiation. One of the analysed proteins, Transgelin-2 (TAGL2, spot 866), was up-regulated 3-fold after 4 days of differentiation. TAGL2 is part of the calponin family of cytoskeleton proteins and is known to directly interact with actin during polymerization. Studies have also suggested that transgelin levels are associated with Ras activation in epithelial cells[3]. Further studies will be required to identify its true function in differentiating NSCs.

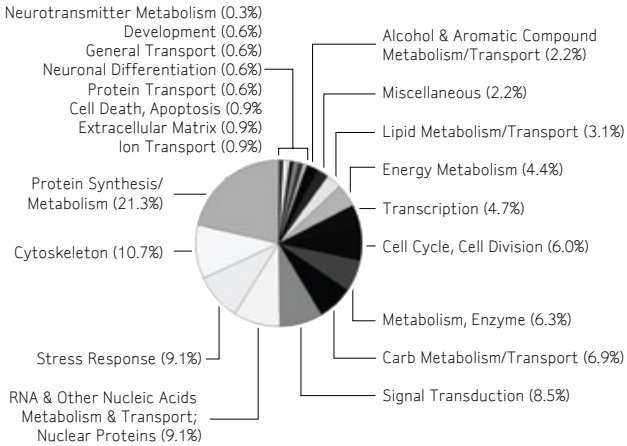

Figure 2. Changes in the relative quantitative distribution of functional protein categories during differentiation of ReNcell VM cells. The 21 functional protein categories was based on n=318 individual proteins and were largely assigned according to gene ontology database.

Summary

In this report we show extensive changes in the proteomic profile of ReNcell VM cells that are associated with terminal differentiation. This is a textbook example of how the ReNcell VM line can be used in combination with proteomics to study human neural differentiation and development *in vitro*. This cell line is easily maintained as a stable proliferating culture and is differentiated upon the removal of growth factors from the culture medium. Convenient culturing and flexible differentiation make ReNcell VM the ideal platform for research and discovery.

References

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