Neurodifferentiation potential of three human embryonic stem cell lines.

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Pluripotent human embryonic stem (hES) cells lines have considerable potential in regenerative medicine and for the production of functional cell substrates for bioassays. Neurons are one lineage of interest; allowing basic studies of cell commitment and development and opportunities to evaluate cell function with the generation of neuronal cell lineages from hES. The search for new therapies for neurodegenerative diseases and basic research into hES lines has resulted in a proliferation of differentiation strategies. This plethora of strategies and limited assessment of them is diluting the impact of these cells thereby limiting their potential in establishing new bioassays. The principal challenges in developing a successful differentiation protocol are: a robust reproducible protocol, which produces high yield of a defined cell population; a minimum period to complete the differentiation process; and a protocol which provides cells with an "expected physiological function", e.g., release and uptake of neurotransmitters or an electrophysiological response. These challenges are compounded by the effect that inherent differences between human embryonic stem cell lines and their response to culture during the maintenance or differentiation conditions have on the differentiation end point.

In this study, three human embryonic stem cells, WT4 (derived at Kings College London), Shef3 and Shef 7 (derived at the University of Sheffield) were subjected to two neurodifferentiation protocols. The two chemically induced differentiation protocols are based on treatment with retinoic acid (RA) or noggin. Retinoic acid protocol is employed for the differentiation of embryonal carcinoma cell line NTERA-2 (1). Since then, many other studies with different cell types such as mesenchymal stem cells and neuroblastoma cell lines, adopted retinoic acid in their neural differentiation protocols. Noggin is bone morphogenic proteins inhibitor that promotes the conversion of ES cells to phenotypically stable neurons (2). Noggin has been mainly employed in stem cells differentiation protocols.

We aim to evaluate the potential of three hES cell lines of normal karyotype, 46 XX or XY, to differentiate into neurons. However, none of these cells were exploited in neurodifferentiation studies. The derivation and culture of two of the cell lines has previously been described, with WT4 being differentiated to macrophages and monocytes (3), Shef3 has been cultured and characterised (4). Differentiation studies have not presently been carried out on the third cell line, Shef7.

A five step differentiation protocol was previously described for other hES lines (5); however, it has not been directly assessed or compared in the lines being evaluated in the current work. The undifferentiated stem cells were grown on mouse feeder layer and expanded by manual passaging (stage 1). The stem cell colonies were enzymatically

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dissociated with collagenase IV to form embryonic bodies (EBs). The EBs were cultured in suspension in hES media for 3 days (stage 2). The embryonic bodies were then plated under adherent conditions and stimulated to differentiate with retinoic acid or noggin in serum free media during 4 days (stage 3). Stimulated cell cultures were incubated under serum free conditions with bFGF for 7 days (stage 4) and for another 7 days with cAMP (stage 5). Following treatment (21 days in total), the neurodifferentiation process was assessed and characterised by phase contrast and epifluorescence microscopy. Differentiated cells resemble morphologically primary neuronal cultures exhibiting elaborate axons and dendrites. Immunoflurescence studies confirmed the presence of MAP2 and βIII-tubulin (neuronal markers) at the stage 5 of the differentiation. Further investigations are now in progress to evaluate what specific neuronal lineages are produced and the electrophysiological potential of differentiated cells.

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