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BRAIN RESEARCH

Research Report

TAT-PAX6 protein transduction in neural progenitor cells: A novel approach for generation of dopaminergic neurones *in vitro*

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ABSTRACT

Neural stem cells (NSCs) have the potential to be used for the treatment of Parkinson's disease (PD), as they can be expanded, manipulated and differentiated *in vitro* to generate dopaminergic neurones which are suitable for transplantation. Since NSCs have a tendency to follow an astrocytic lineage after differentiation *in vitro*, researchers are investigating ways to induce a neuronal phenotype in these cells. In this study, the human immunodeficiency virus 1 (HIV-1) transactivator of transcription (TAT) protein transduction domain (PTD) system was used in an attempt to promote neuronal differentiation in rodent NSCs. A fusion protein that incorporated both the TAT PTD and the Pax6 protein (a determinant of neurogenesis) was created and added to the differentiation phase of embryonic day (E) 12 rat ventral mesencephalic (VM) neurosphere cultures. Subsequently, application of dopaminergic growth factors (GFs) was used in an attempt to induce the newly-generated neuronal progenitors to adopt a dopaminergic phenotype. In addition, a technique involving the differentiation of intact neurospheres (instead of the differentiation of neurosphere-derived dissociated cells) was investigated for its ability to promote dopaminergic neurogenesis. Immunocytochemical analysis of the differentiated neurosphere cultures indicated that both of these techniques had a significant effect on the emergence of dopaminergic neurones. Moreover, upon combination of these techniques, a further increase in dopaminergic neuronal generation was observed. Based on the findings of the present study, it is clear that NSCs are greatly influenced by their environment and that optimised *in vitro* conditions can support the potential of these cells to differentiate into dopaminergic neurones.

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Abbreviations: 6-OHDA, 6-hydroxydopamine; Antp, *Drosophila* antennapedia homeotic transcription factor; bFGF, basic fibroblast growth factor; CRL, crown-rump length; E, embryonic day; EGF, epidermal growth factor-like growth factor; FGF8, fibroblast growth factor 8; GDF5, growth/differentiation factor 5; GDNF, glial cell line-derived factor; GF(s), growth factor(s); GFAP, glial fibrillary acidic protein; GFP, green fluorescent protein; HA, hemagglutinin; HIV-1, human immunodeficiency virus 1; HSV-1, herpes simplex virus 1; MPP+, *N*-methyl-4-phenylpyridium; NSC, neural stem cell; PD, Parkinson's disease; PTD, protein transduction domain; SHH, sonic hedgehog; SN, substantia nigra; SVZ, subventricular zone; TAT, transactivator of transcription; TH, tyrosine hydroxylase; VM, ventral mesencephalon

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1. Introduction

The degeneration of dopaminergic neurones in the substantia nigra (SN) of the midbrain is the pathological hallmark of Parkinson's disease (PD). Since this degeneration is confined to a specific brain area and cell type, much research has focused on developing cell replacement therapies for the disease. Neural stem cells (NSCs) have been investigated as a potential source of dopaminergic neurones for transplantation in PD, since these cells can be expanded, manipulated and differentiated *in vitro* as 'neurosphere' cultures.

In recent years, protein transduction domains (PTDs) have emerged as attractive biological delivery tools. The term 'PTD' describes small cationic peptides, of approximately 10–16 amino acids in length, which have the ability to traverse biological membranes independent of receptor- or endocytosis-mediated mechanisms (Dietz and Bahr, 2004; Snyder and Dowdy, 2004). Examples of proteins incorporating PTDs include the human immunodeficiency virus (HIV)-1 transactivator of transcription (TAT) protein, the herpes simplex virus (HSV)-1 DNA-binding protein VP22, and the *Drosophila* antennapedia (Antp) homeotic transcription factor. To date, an assortment of recombinant PTD-containing proteins has been generated and used to facilitate the transportation of a range of cargos into a variety of cell types. The TAT system has been investigated in relation to a broad range of disease treatments including diabetes (Klein et al., 2004), breast cancer (Shokolenko et al., 2005), multiple

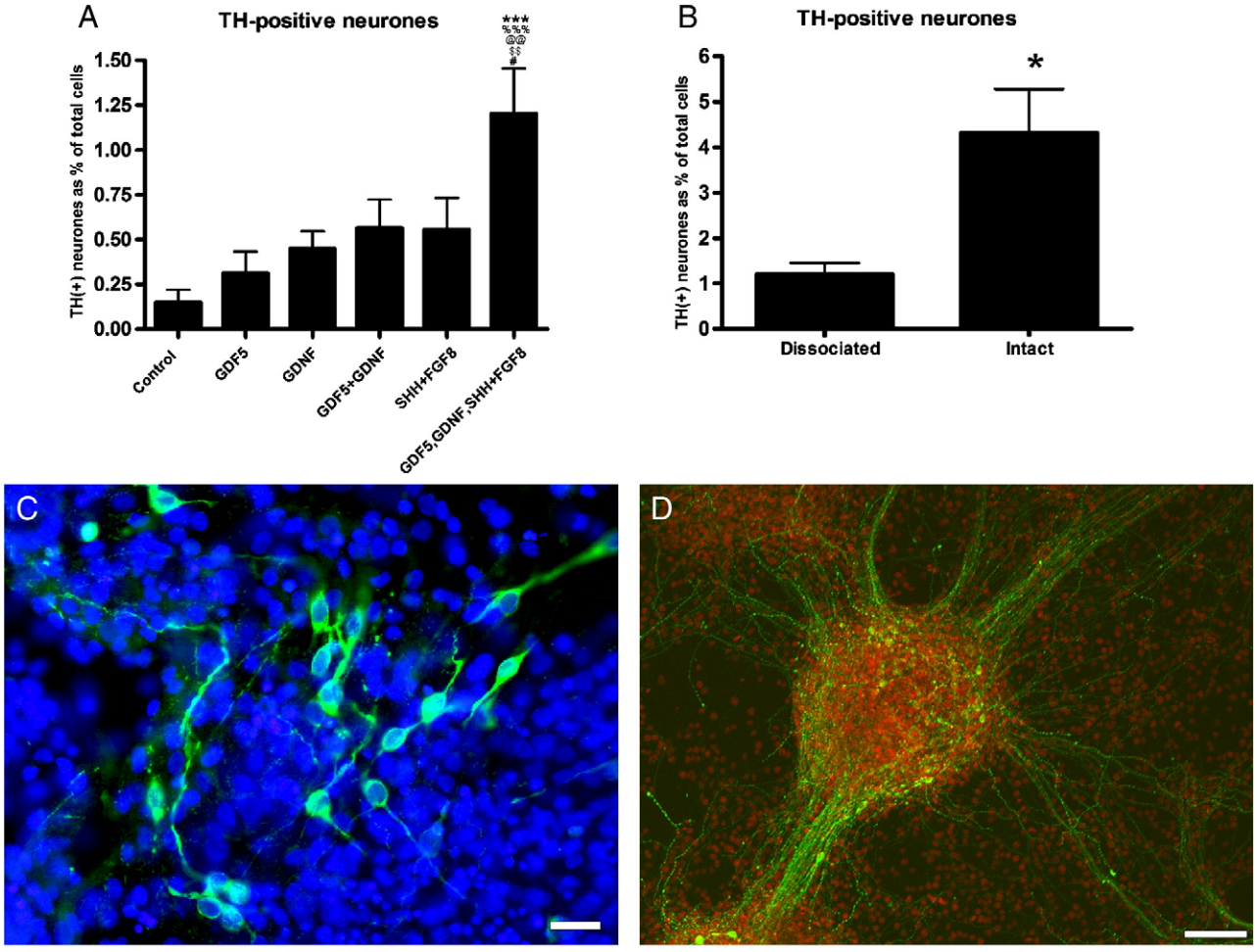

Fig. 1 – (A) Yields of TH-positive neurones in differentiated E12 rat VM neurosphere cultures after treatment with growth factors. Data represent mean ± SEM from three independent experiments. $^{***}P<0.001$ vs. controls; $^{\%\%\%}P<0.001$ vs. GDF5; $^{@@}P<0.01$ vs. GDNF; $^{\$\$}P<0.01$ vs. SHH + FGF8; and $^{\#}P<0.05$ vs. GDF5 + GDNF, ANOVA with post-hoc Tukey's test. (B) Yields of TH-positive neurones in E12 rat VM neurosphere cultures, differentiated as dissociated or intact neurospheres, after treatment with GDF5 + GDNF + SHH + FGF8. Data represent mean ± SEM from three independent experiments. $^{*}P<0.05$ vs. the dissociated neurosphere condition; Student's t-test for independent means. (C) Photomicrograph showing a representative differentiated E12 rat VM dissociated neurosphere culture after treatment with GDF5 + GDNF + SHH + FGF8, immunocytochemically stained for TH (green) and counterstained with Hoechst 33258 (blue). Scale bar = 30 μm. (D) Photomicrograph showing a representative differentiated E12 rat VM intact neurosphere culture after treatment with GDF5 + GDNF + SHH + FGF8, immunocytochemically stained for TH (green) and counterstained with PI (red). This photomicrograph represents a merged and projected Z-series of confocal images through a single neurosphere. Scale bar = 100 μm.