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Direct conversion of human fibroblasts into dopaminergic neural progenitor-like cells using TAT-mediated protein transduction of recombinant factors

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ABSTRACT

Recent progress in the generation of induced neural progenitor cells (iNPCs) holds tremendous potential for regenerative medicine. However, a major limitation is the lack of a reliable source for cell replacement therapy in neurological diseases such as Parkinson's disease (PD). Here, we show that the combination of small molecules (SM) and TAT-mediated protein transduction of SOX2 and LMX1a in a 3D sphere culture directly convert human fibroblasts to induced dopaminergic neural progenitor-like cells (iDPCs). The generated iDPCs expressed various NPC markers (*SOX2*, *PAX6*, *NESTIN*, *OLIG2*) and midbrain progenitor markers (*EN1*, *LMX1a*, *FOXA2*, *WNT1*) as detected by immunostaining and real-time PCR. Following differentiation, the majority of cells expressed neuronal dopaminergic markers as indicated by co-expression of TH with NURR1, and/or PITX3. We found that SOX2 and LMX1a TAT-mediated protein transduction in the combination of SM could directly convert human fibroblasts to self-renewal iDPCs. In conclusion, to our best knowledge, this is the first report of generation of safe DPCs and may suggest an alternative strategy for cell therapy for the treatment of neurodegenerative disorders.

1. Introduction

Parkinson's disease (PD) is one of the most common age-related neurodegenerative diseases caused by dopamine-producing neurons in the midbrain substantia nigra. This neurodegenerative disease leads to progressive degeneration affecting millions of people worldwide [1]. To date, there is no definite cure for PD and cell replacement therapy aims to replace and repopulate the lost dopaminergic neurons (DA) with new ones. However, there is still a pressing need to identify an ideal cell source which is patient specific, easy to obtain and expandable with controlled differentiation. Stem cells have the capability to generate desired derivatives to treat these disorders [2]. In recent years, progress in reprogramming and transdifferentiation has led to the proposal of a potential patient-specific cell source for the treatment of a broad range of human neurological disorders (for review see Ref. [3]). These induced DA (iDA) have been generated directly from different mouse and human somatic cell types by ectopic expression of key transcription factors [4–8]. However, these terminally differentiated cells are not proliferative post-mitosis and inadequate for transplantation-based treatments that require large cell numbers. In this respect, hopes have been raised by the generation of induced neural progenitor cells (iNPCs) due to their ability to provide large amounts of cells once reprogrammed and their capability to give rise to differentiated cell progenies *in vitro* and *in vivo* for clinical applications. Currently, several independent groups have generated iNPCs from mouse and human somatic cells by potentially

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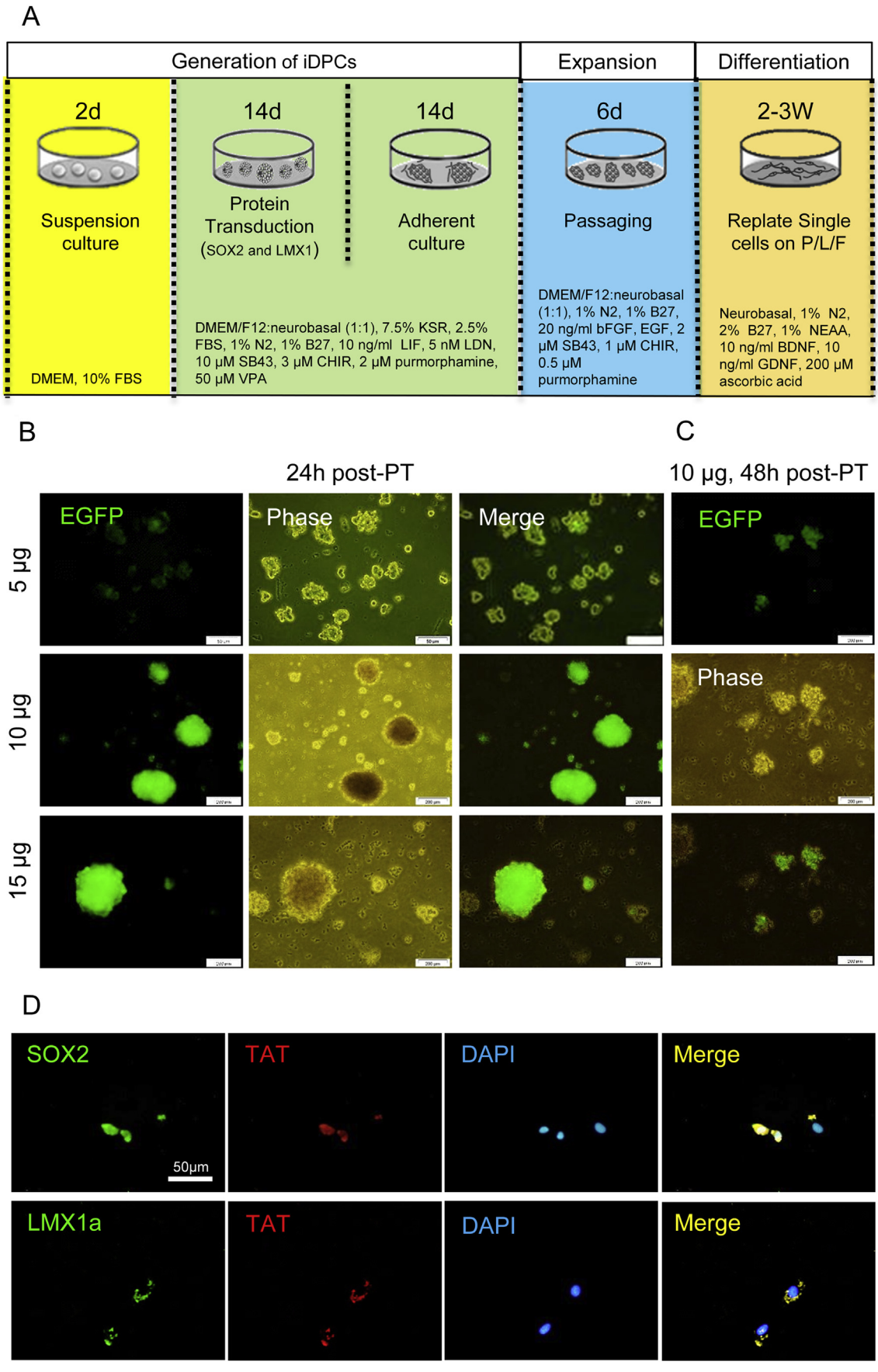
A
Generation of iDPCs
Expansion
Differentiation
2d
14d
14d
6d
2-3W
Suspension culture
Protein Transduction (SOX2 and LMX1)
Adherent culture
Passaging
Replate Single cells on P/L/F
DMEM, 10% FBS
DMEM/F12:neurobasal (1:1), 7.5% KSR, 2.5% FBS, 1% N2, 1% B27, 10 ng/ml LIF, 5 nM LDN, 10 μM SB43, 3 μM CHIR, 2 μM purmorphamine, 50 μM VPA
DMEM/F12:neurobasal (1:1), 1% N2, 1% B27, 20 ng/ml bFGF, EGF, 2 μM SB43, 1 μM CHIR, 0.5 μM purmorphamine
Neurobasal, 1% N2, 2% B27, 1% NEAA, 10 ng/ml BDNF, 10 ng/ml GDNF, 200 μM ascorbic acid
B
24h post-PT
C
10 μg, 48h post-PT
5 μg
10 μg
15 μg
EGFP
Phase
Merge
EGFP
Phase
D
SOX2
TAT
DAPI
Merge
50μm
LMX1a
TAT
DAPI
Merge