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Enhancing oligodendrocyte differentiation by transient transcription activation via DNA nanoparticle-mediated transfection

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ABSTRACT

Current approaches to derive oligodendrocytes from human pluripotent stem cells (hPSCs) need extended exposure of hPSCs to growth factors and small molecules, which limits their clinical application because of the lengthy culture time required and low generation efficiency of myelinating oligodendrocytes. Compared to extrinsic growth factors and molecules, oligodendrocyte differentiation and maturation can be more effectively modulated by regulation of the cell transcription network. In the developing central nervous system (CNS), two basic helix-loop-helix transcription factors, Olig1 and Olig2, are decisive in oligodendrocyte differentiation and maturation. Olig2 plays a critical role in the specification of oligodendrocytes and Olig1 is crucial in promoting oligodendrocyte maturation. Recently viral vectors have been used to overexpress Olig2 and Olig1 in neural stem/progenitor cells (NSCs) to induce the maturation of oligodendrocytes and enhance the remyelination activity *in vivo*. Because of the safety issues with viral vectors, including the insertional mutagenesis and potential tumor formation, non-viral transfection methods are preferred for clinical translation. Here we report a poly(β-amino ester) (PBAE)-based nanoparticle transfection method to deliver Olig1 and Olig2 into human fetal tissue-derived NSCs and demonstrate efficient oligodendrocyte differentiation following transgene expression of Olig1 and Olig2. This approach is potentially translatable for engineering stem cells to treat injured or diseased CNS tissues.

Statement of Significance

Current protocols to derive oligodendrocytes from human pluripotent stem cells (hPSCs) require lengthy culture time with low generation efficiencies of mature oligodendrocytes. We described a new approach to enhance oligodendrocyte differentiation through nanoparticle-mediated transcription modulation. We tested an effective transfection method using cell-compatible poly (β-amino ester) (PBAE)/DNA nanoparticles as gene carrier to deliver transcription factor Olig1 and Olig2 into human fetal tissue-derived neural stem/progenitor cells, and showed efficient oligodendrocyte differentiation following transgene expression of Olig1 and Olig2. We believe that this translatable approach can be applied to many other cell-based regenerative therapies as well.

1. Introduction

Existent approaches of directed differentiation require extended exposure of human pluripotent stem cells (hPSCs) to

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extrinsic factors, such as growth factors and small molecules; therefore they are challenging to implement for transplanted hPSCs *in vivo* [1–4]. Specifically, current protocols to derive oligodendrocytes from hPSCs are limited in application because of lengthy culture time required (80 to 200 days) and low generation efficiencies of mature oligodendrocytes [3–6]. There is an urgent need to develop more efficient methods to accelerate the differentiation and maturation timeline of hPSCs for regenerative therapy.

In comparison with the extrinsic factors supplemented in the medium, stem cell differentiation and maturation can be more efficiently modulated through regulating intrinsic factor expression, such as resetting the transcription network using transcription factors [7,8]. In the developing central nervous system (CNS), two basic helix-loop-helix (bHLH) transcription factors, Olig1 and Olig2, are expressed in oligodendrocyte progenitor cells and myelinating oligodendrocytes; Olig2 is decisive for the specification of oligodendrocytes and Olig1 is essential in fostering oligodendrocyte differentiation and subsequent myelination primarily in the brain [9,10]. Overexpression of Olig2 in neural stem/progenitor cells (NSCs) by viral vector has shown to promote oligodendrocyte differentiation and maturation and enhance remyelination activity *in vivo* [11,12].

Currently viral vectors have been extensively used to mediate transfection of transcription factors to stem cells to control their differentiation and maturation [13]. However, these viral vectors have raised lots of safety concerns with the insertional mutagenesis and excessive inflammation and immune response [14]. Viral vector-mediated persistent expression of exogenous transcription factors may unfavorably affect the differentiated cell maturation and function [15,16].

Numerous biomaterials have been investigated as potential non-viral gene delivery vectors [17–20]. As compared to viral vectors, biomaterial-based vectors are easier to manufacture and scale-up, but they are less efficient in mediating transgene expression. In particular, poly (β-amino ester)s (PBAEs) have been studied as polymeric gene carriers due to their structural versatility, biodegradability, and low cytotoxicity [21–24]. PBAEs have shown to condense plasmid DNA forming nanoparticles with relatively high transgene expression in several stem cell types [21,25,26]. Here we develop an efficient approach to expedite and enhance

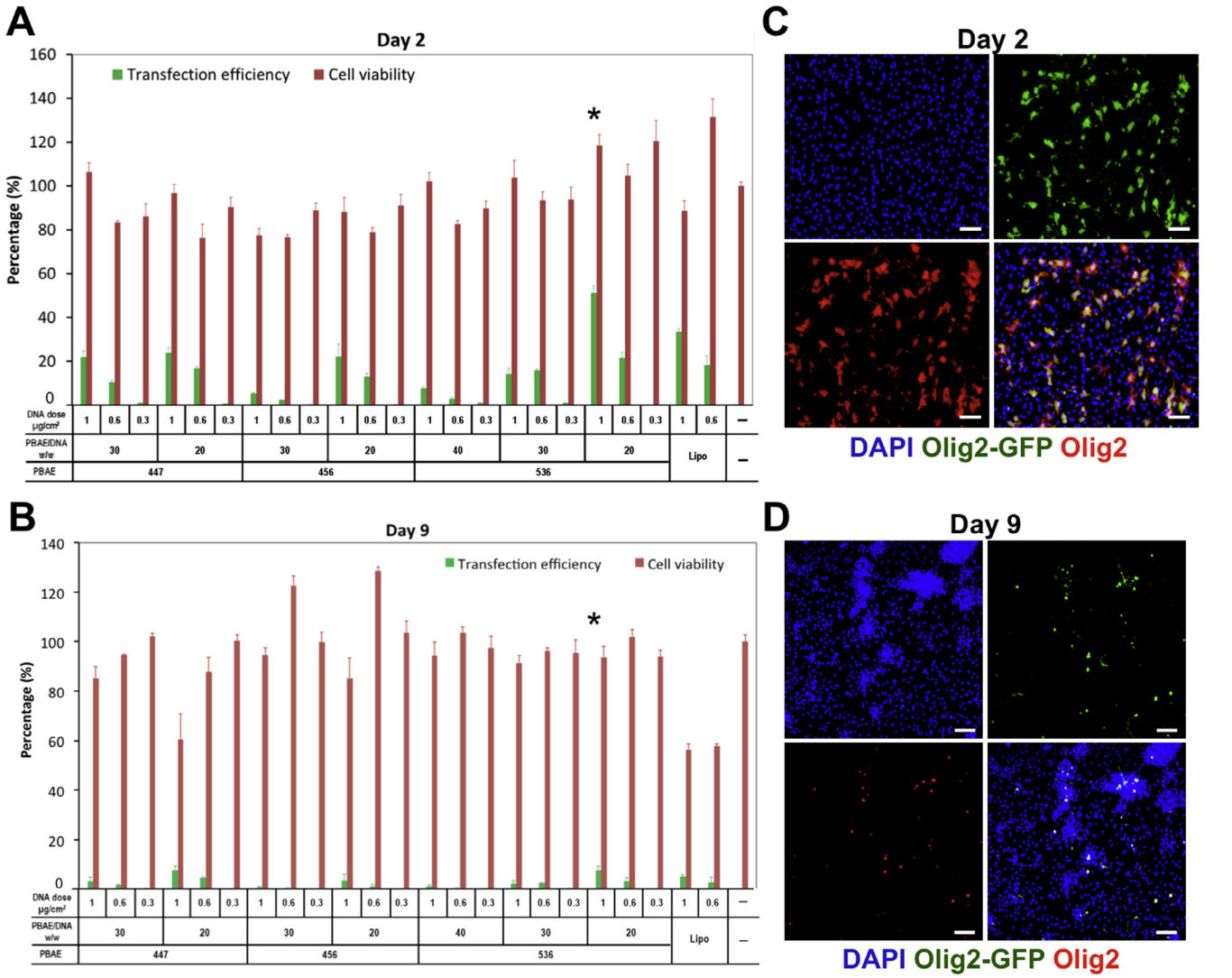

Fig. 1. Identification of a nanoparticle composition with high transfection efficiency and low cytotoxicity. (A and B) Initial screening used Olig2-GFP plasmid DNA doses of 0.3, 0.6, and 1 μg/cm^2 and an abbreviated range of PBAE/plasmid DNA ratios (20, 30, and 40 w/w) in order to identify top polymers among 447, 456, and 536 based on their transfection efficiencies and cytotoxicities on days (A) 2 and (B) 9. (C and D) Expression of transfected Olig2-GFP (green) and Olig2 (red) on days (C) 2 and (D) 9 of the tested PBAE 536 at the polymer/DNA ratio of 20 w/w and the DNA dose of 1 μg/cm^2. Extensive colocalization of GFP and Olig2 were observed on days 2 and 9. DAPI was used to stain cell nuclei in blue. Scale bar = 100 μm.