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

Xiaowei Li [a,b,c], Stephany Y. Tzeng [a,c,d], Camila Gadens Zamboni [a,d], Vassilis E. Koliatsos [e,f,g], Guo-li Ming [f,g,h,i], Jordan J. Green [a,b,c,d], Hai-Quan Mao [a,b,c,*]

[a] Translational Tissue Engineering Center, Johns Hopkins School of Medicine, Baltimore, MD 21287, USA
[b] Department of Materials Science & Engineering, Johns Hopkins University, Baltimore, MD 21218, USA
[c] Institute for NanoBioTechnology, Johns Hopkins University, Baltimore, MD 21218, USA
[d] Department of Biomedical Engineering, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA
[e] Department of Pathology, Division of Neuropathology, Johns Hopkins School of Medicine, Baltimore, MD 21287, USA
[f] Department of Neurology, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA
[g] Department of Psychiatry & Behavioral Sciences, Johns Hopkins School of Medicine, Baltimore, MD 21287, USA
[h] Institute for Cell Engineering, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA
[i] The Solomon H. Snyder Department of Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA

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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

* Corresponding author at: Johns Hopkins University, 205 Maryland Hall, 3400 N. Charles Street, Baltimore, MD 21218, USA.
E-mail address: hmao@jhu.edu (H.-Q. Mao).

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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

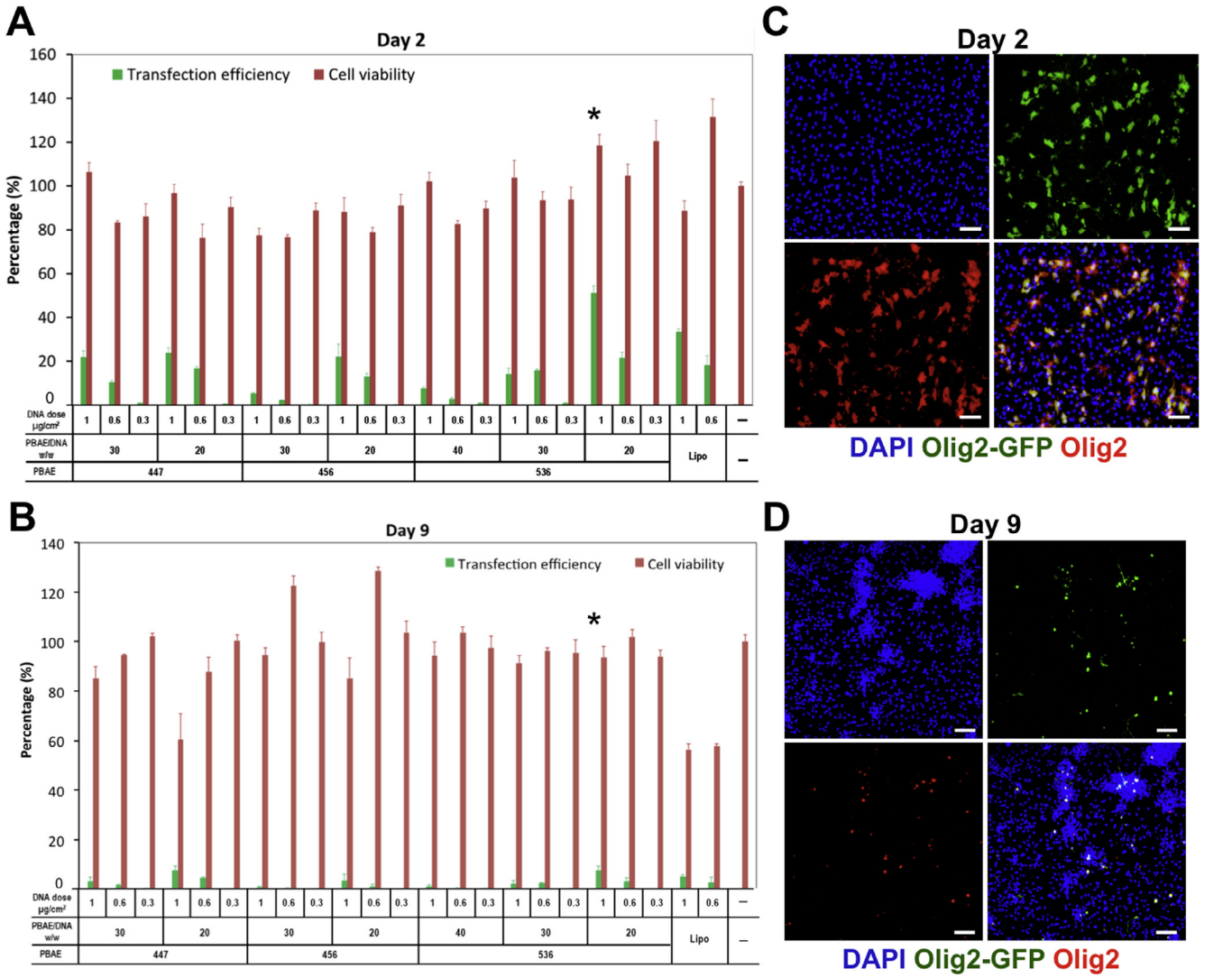

Fig. 1. Identification of a nanoparticle composition with high transfection efficiency and low cytotoxicity. (A and B) Initial screening used Olig2-GFP plasmid DNA doses of 0.3, 0.6, and 1 μg/cm^2 and an abbreviated range of PBAE/plasmid DNA ratios (20, 30, and 40 w/w) in order to identify top polymers 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.

oligodendrocyte differentiation from human fetal tissue-derived NSCs through PBAE-DNA nanoparticle-mediated transient expression of Olig1 and Olig2 in hNSCs.

2. Results and discussion

2.1. Highly efficient PBAE-DNA nanoparticle-mediated transfection of hNSCs

A series of PBAE polymers were synthesized following the method that we have previously reported using the monomers and the reaction scheme shown in Fig. S1 [21,22]. Briefly, a diacrylate backbone (B), an amino-alcohol side chain (S), and an amine containing end-capping (E) were conjugated through a two-step process in which the addition of the end-group followed the formation of a BS base-polymer. Polymers were named according to their "BSE" structure, where monomers forming the base-polymer BS were identified by the number of carbons in its hydrocarbon portion. For example, 536 refers to the polymer synthesized with B5, S3, and E6, where B5 corresponds to a backbone with 5 hydrocarbons between the acrylate groups and S3 to a side chain with 3 hydrocarbons between the amine and alcohol groups. The

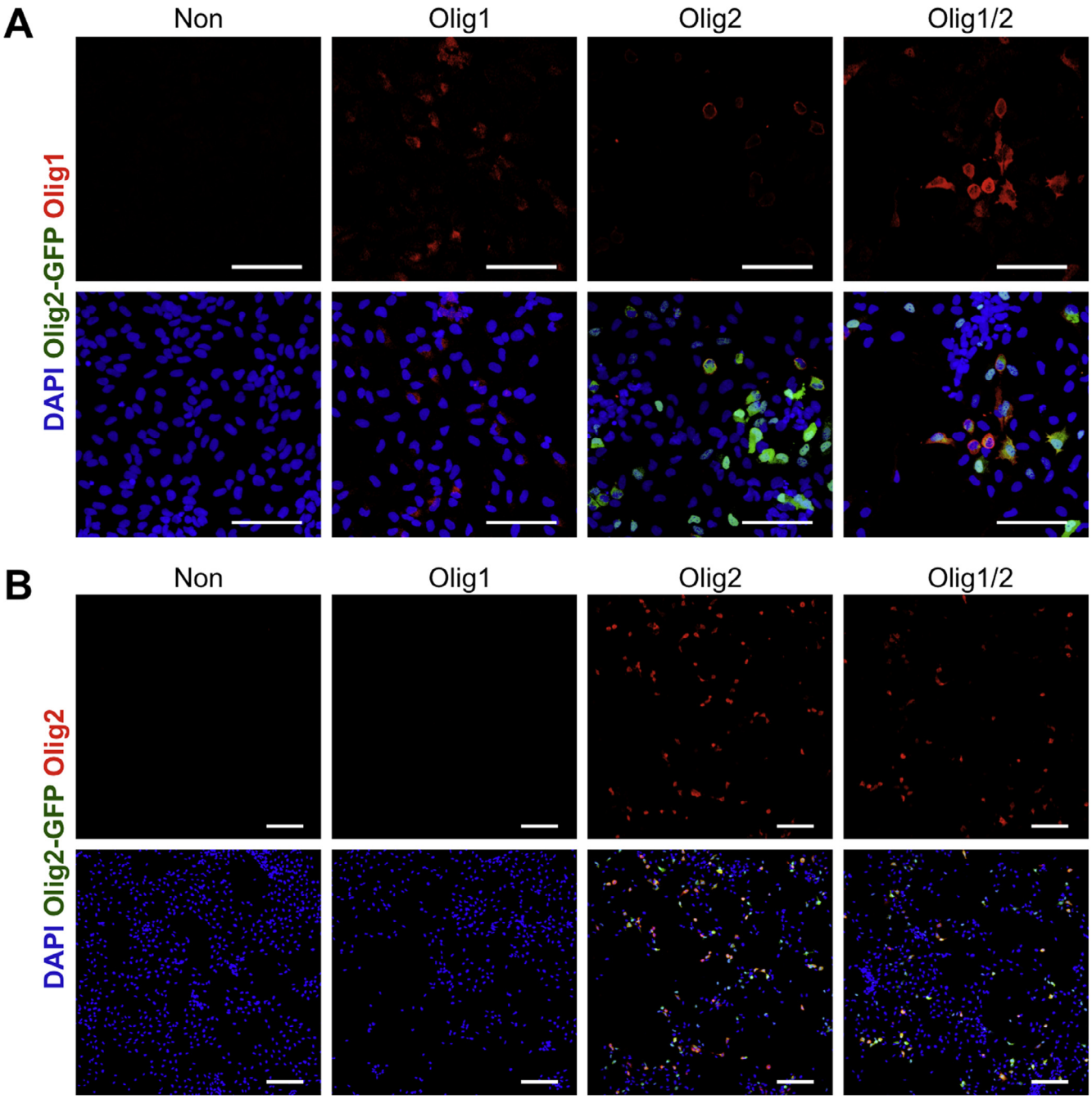

Fig. 2. Nanoparticle-mediated Olig1 and Olig2 expression on hNSCs. (A) Olig1 (red) expression in hNSCs after transfection with PBAE 536 nanoparticles containing plasmid encoding Olig1, plasmid encoding Olig2-GFP, or the mixture of these two plasmids (Olig1/2; 0.5 μg/cm^2 Olig1 and 0.5 μg/cm^2 Olig2-GFP, respectively) at the polymer/DNA ratio of 20 w/w and the DNA dose of 1 μg/cm^2 on day 2. (B) Olig2 (red) expression in hNSCs after transfection with PBAE 536 nanoparticles containing plasmid encoding Olig1, plasmid encoding Olig2-GFP, or the mixture of these two plasmids on day 2. Non-transfected cells were used as a control. DAPI was used to stain cell nuclei in blue. Scale bar = 100 μm.

numbers assigned for end-capping monomers are merely sequential, arranged according to structural similarities among amine groups.

As previously demonstrated by us, single changes on the hydrocarbon content, and therefore hydrophobicity, of the BS base-polymer can significantly modify the polymer activity [21,22]. Increase in PBAE hydrophobicity is associated with high gene expression, but only up to a certain limit, from which the increase in cytotoxicity becomes much higher than any additional increment in transfection efficiency. On the other hand, a balanced hydrophobicity/hydrophilicity between backbone and side chain monomers can afford high transfection efficiencies while preserving cell viability. In other words, optimized transfection and viability outcomes can be achieved, for example, by employing hydrophobic backbones (e.g. B5) combined with hydrophilic side chains (S3), or yet, intermediately-hydrophobic base diacrylates (e.g. B4) with hydrophobic side chains (S5). Further end-modification of PBAE polymers with secondary (E6) or tertiary amines (E7) groups enables high endosomal buffering, as opposed to the low buffering effect of primary amines (e.g. E4). High buffering capacity is a desirable characteristic, since it is necessary, even though not enough, to favor transfection efficiency [27]. Based on such findings and previous work with glial cells [21,22], three top-performing PBAE formulations (447, 456, and 536) were chosen to achieve an optimal transfection condition with high level of transgene expression and low cytotoxicity for hNSCs.

Initial screens used an Olig2-GFP plasmid DNA dose of 0.3, 0.6, and 1 μg/cm^2 and a selected range of PBAE/plasmid DNA ratios of 20, 30, and 40 w/w in order to identify top polymers from a group consisting PBAE 447, 456, and 536 (Fig. 1). Among all condition tested, PBAE 536 at the polymer/DNA ratio of 20 w/w with the DNA dose of 1 μg/cm^2, showed the highest transfection efficiency of 57.4 ± 2.4% and cell viability of 118.3 ± 5.1% relative to non-transfected cells on day 2 following transfection (Fig. 1A). The transfected gene expression lasted for at least 10–14 days, and showed efficiency of 8.2 ± 0.6% with a cell viability of 93.6 ± 4.5% relative to non-transfected cells on day 9 (Fig. 1B). Immunostaining analysis confirmed the expression of GFP was co-localized to Olig2 expression in the transfected hNSCs on both days 2 and 9 (Fig. 1C and D). We have chosen PBAE 536 at the polymer/DNA ratio of 20 w/w and the DNA dose of 1 μg/cm^2 for following studies.

2.2. Nanoparticle-mediated co-delivery of Olig1 and Olig2 into hNSCs

Through the optimized transfection condition identified above, we successfully delivered Olig1, Olig2, and both Olig1 and Olig2 into hNSCs through nanoparticles containing plasmid encoding Olig1, plasmid encoding Olig2, and the mixture of these two plasmids (Olig1/2; 0.5 μg/cm^2 Olig1 and 0.5 μg/cm^2 Olig2-GFP), respectively (Fig. 2). Olig1 and Olig2 were transfected into hNSCs with an efficiency of 36.7 ± 6.8% and 33.3 ± 7.0%, respectively. Co-delivery of Olig1 and Olig2 was shown to be feasible through incorporation of these two plasmids into a single dose of PBAE nanoparticles. Olig1 and Olig2 were expressed in the Olig1/2-transfected hNSCs with the efficiencies of 31.5 ± 6.5% and 35.5 ± 7.0%, respectively. About 22.5 ± 5.0% cells simultaneously expressed Olig1 and Olig2 according to the co-localization of Olig1 and Olig2 in nuclei. Around 9.0 ± 3.0% cells only expressed Olig1 while 13.0 ± 5.0% cells only expressed Olig2.

We investigated the effect of Olig1 and Olig2 expression on the long-term viability of hNSCs using AlamarBlue assay (Fig. 3). On day 15, Olig1, Olig2, and Olig1/2- transfected cells showed viabilities of 102.8 ± 4.3%, 105.6 ± 4.8%, and 110.2 ± 5.6%, respectively, relative to non-transfected cells on day 2. There was no significant cell death following the transfection for 15 days. In addition, since the cells in all groups were cultured under an oligodendrocyte enrichment medium with a cocktail of growth factors including PDGF-AA, NT-3, and FGF-2, all of these cells did not show significant proliferation after day 5. These cells may prefer differentiation rather than proliferation due to the differentiation culture condition.

2.3. Enhancing oligodendrocyte differentiation by nanoparticle-mediated Olig1 and Olig2 expression

We have previously demonstrated that multiple-round transfections could enhance the expression of transcription factor, such as neurogenin 2 (Ngn2), in human fetal tissue-derived NSCs to improve their neuronal differentiation and maturation *in vitro* and at a brain lesion site after traumatic injury [21]. In this study, we followed the similar protocol and performed 4 rounds of transfection in hNSCs on Days 2, 13, 20, and 27, respectively, to extend the expressions of Olig1 and Olig2 (Fig. 4A). We did not observe significant difference in term of transfection efficiency at different times following transfection.

First we examined the differential gene expression of hNSCs following transfection with Olig1, Olig2, or Olig1/2 through quantitative real-time PCR. Non-transfected cells were used as a control. As shown in Fig. 4B, a specific gene for early oligodendrocyte progenitor cells (OPCs), platelet-derived growth factor receptor alpha (PDGFRα), was upregulated following Olig1, Olig2, and Olig1/2 transfection in hNSCs on day 4. Higher levels of PDGFRα were also observed in three transfected groups on day 27. One specific gene for late OPCs, 2, 3-cyclic nucleotide 3 phosphodiesterase (CNP), was also upregulated following Olig1, Olig2, and Olig1/2 transfection in hNSCs on day 4. While one of specific genes for myelinating oligodendrocyte, myelin oligodendrocyte glycoprotein (MOG) was enriched only in the Olig1/2-transfected cells on day 35. In addition, lower levels of nestin gene expression were observed in three transfected groups compared to the control group on both days 4 and 11. In term of neurogenic gene, Tuj1, and astrocytic gene, glial fibrillary acidic protein (GFAP), their expressions did not show clear trends among all four groups.

We then characterized cell phenotypes through immunostaining on both days 20 and 35. As shown in Fig. 5, no significant differences were detected among all the groups in term of Tuj1+ neurons and GFAP+ astrocytes on day 20. In consideration of the

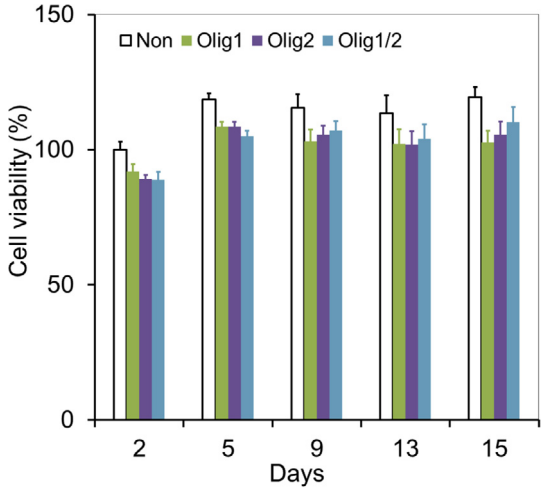

Fig. 3. Long-term viability of hNSCs after nanoparticle-mediated transfection. Viabilities of Olig1, Olig2, or Olig1/2-transfected hNSCs were inspected by AlamarBlue assay on days 2, 5, 9, 13, and 15, respectively (n = 4; $^*P < 0.05$). Cells without transfection were used as a control. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

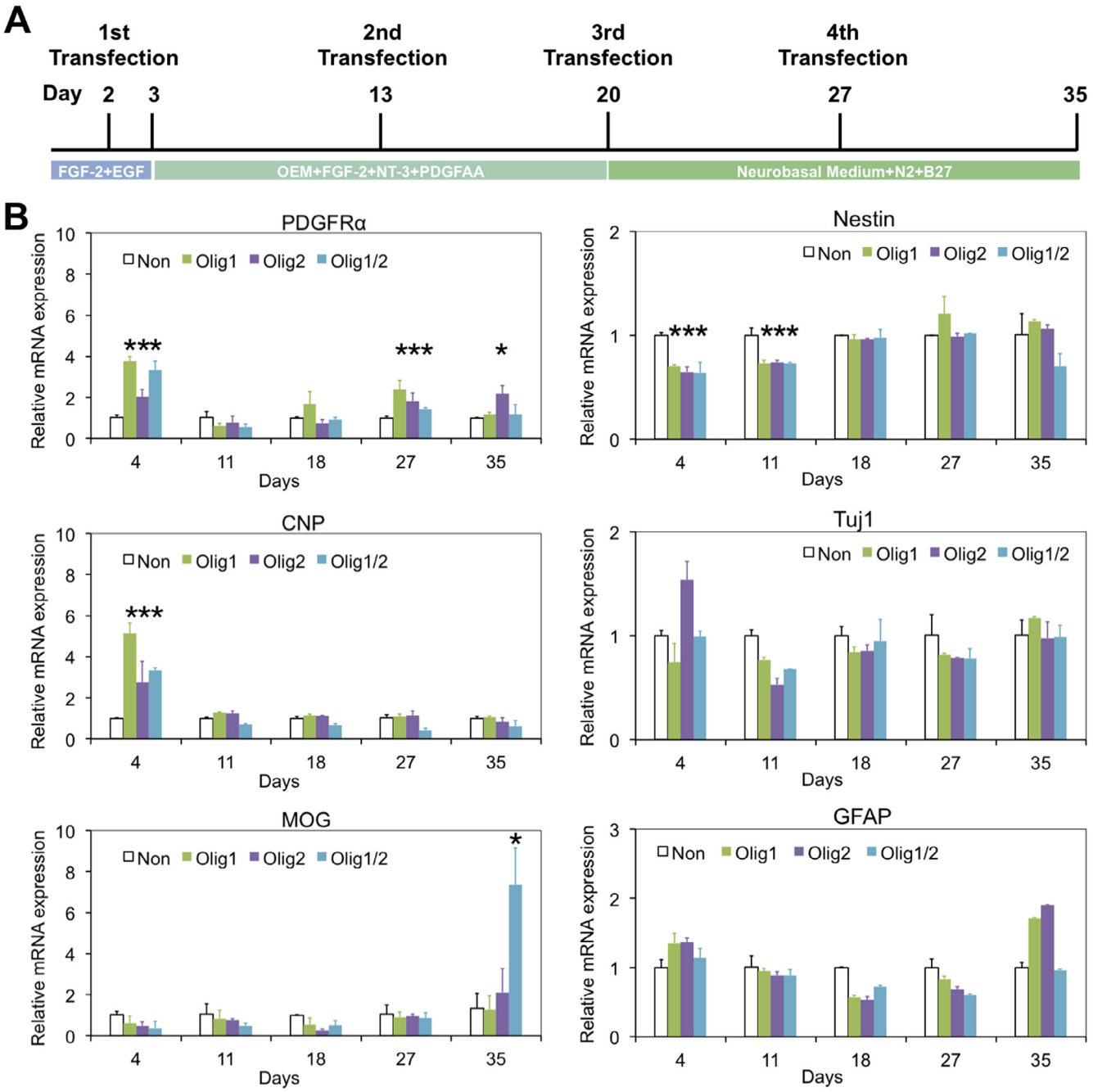

Fig. 4. Modulating oligodendrocyte differentiation from hNSCs through nanoparticle-mediated expression of Olig1 and Olig2. (A) Schematic of oligodendrocyte differentiation from hNSCs. Nanoparticle-mediated transfection was performed on days 2, 13, 20, and 27, respectively. (B) PDGFRα, CNP, MOG, nestin, Tuj1, and GFAP expression of Olig1, Olig2, and Olig1/2-transfected hNSCs on days 4, 11, 18, 27, and 35, respectively, investigated by quantitative real-time PCR assay. Cells without transfection were used as a control (n = 3; $^{*}P < 0.05$).

oligodendrocyte lineage, approximately 30% cells in three transfected groups were bipolar PDGFRα+ OPCs, which were significantly more than those in the control group (Olig1/2: 31.7 ± 7.5%, Olig1: 23.4 ± 4.7%, Olig2: 29.7 ± 6.0%, and Non: 9.2 ± 1.4%; n = 6; $P < 0.05$). There were no significant differences in PDGFRα+ OPCs observed among Olig1, Olig2, and Olig1/2 transfected groups. Whereas the Olig1/2-expressing hNSCs produced the largest number of O4+ pre-myelinating oligodendrocytes with branched processes among all the groups on day 20 (Olig1/2: 23.8 ± 4.8%, Non: 4.8 ± 1.1%, Olig1: 8.9 ± 1.8%, and Olig2: 15.9 ± 3.2%; n = 6; $P < 0.05$). These results imply that nanoparticle-mediated Olig expression may expedite hNSC differentiation into OPCs and the combination of Olig1 and Olig2 may enhance oligodendrocyte differentiation more effectively than Olig1 or Olig2 only.

After culture in a growth factor-free differentiation medium for additional two weeks, O4+ pre-myelinating oligodendrocytes displayed a significant increase by 3.2-fold, 3.2-fold, and 1.8-fold in Olig1, Olig2, and Olig1/2 transfected groups, respectively (Fig. 6, n = 6; $P < 0.05$). Approximately 44% O4+ oligodendrocytes appeared in the Olig1/2-transfected group on day 35. In contrast, in the control group, only less than 5% O4+ cells appeared on day 35, which was similar to those on day 20 (4.8 ± 1.0% *vs.* 4.9 ± 1.0%; n = 6). There were about 50% PDGFRα+ OPCs in the control group with 5-fold increase from day 20 to day 35 (52.2 ± 10.4%

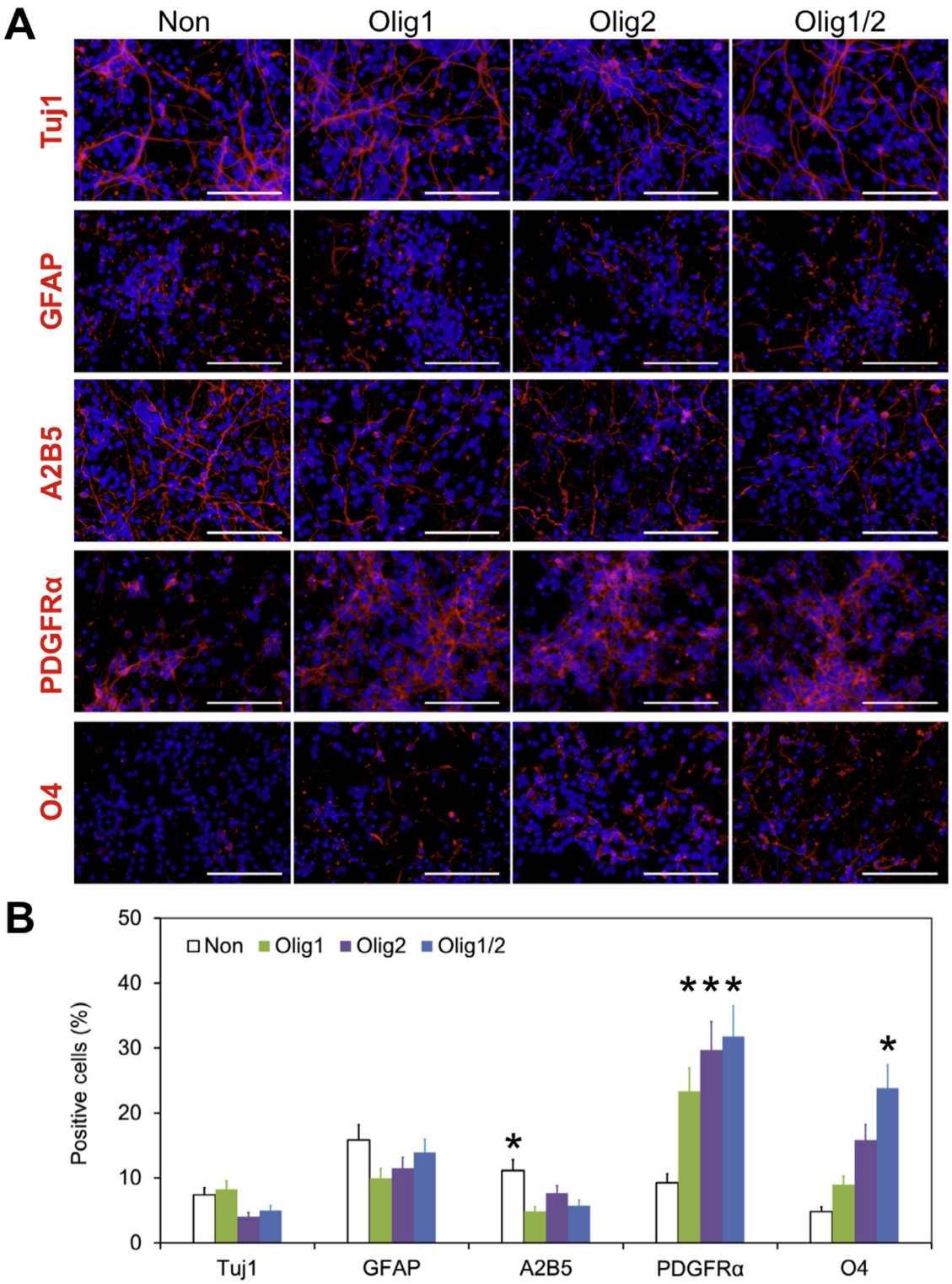

Fig. 5. Enhancing oligodendrocyte differentiation by nanoparticle-mediated expression of Olig1 and Olig2. (A) Differentiation of Olig1, Olig2, and Olig1/2-transfected hNSCs on day 20. Cells were stained with Tuj1 (red), GFAP (red), A2B5 (red), PDGFRα (red), and O4 (red), respectively. Cells without transfection were used as a control. DAPI was used to stain cell nuclei in blue. Scale bar = 100 μm. (B) Quantitative analysis of the differentiation of hNSCs on day 20 ($^{*}P < 0.05$). The positive cells for each marker were counted. At least 6 random fields per sample were taken for quantitative analysis.

vs. to 9.2 ± 1.4%; n = 6; $P < 0.05$). There was no significant difference among all the groups in term of PDGFRα+ cells on day 35. These results confirm that nanoparticle-mediated Olig1 and Olig2 expression can expedite the differentiation of hNSCs into O4+ oligodendrocytes.

Additionally, about 25% cells in the control group are Tuj1+ neurons, which were significantly more than those in three transfected groups on day 35 (Non: 24.7 ± 4.9%, Olig1: 2.8 ± 1.0%, Olig2: 4.9 ± 2.0%, and Olig1/2: 1.8 ± 1.0%; n = 6; $P < 0.05$). Western blot analysis also confirmed the highest level of Tuj1 in the control

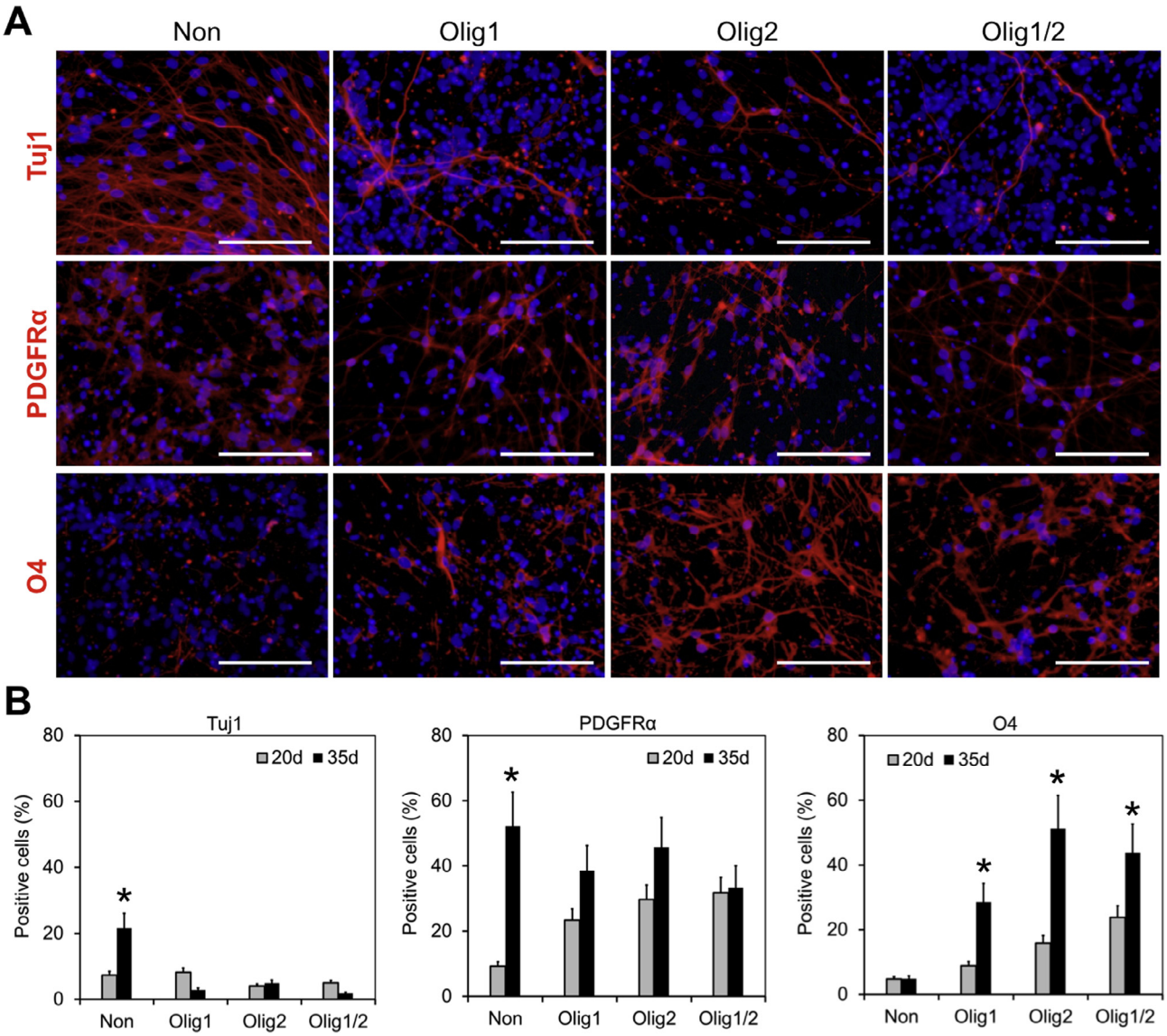

Fig. 6. Expediting oligodendrocyte differentiation by nanoparticle-mediated expression of Olig1 and Olig2. (A) Differentiation of Olig1, Olig2, and Olig1/2-transfected hNSCs on day 35. Cells were stained with Tuj1 (red), PDGFRα (red), and O4 (red), respectively. Cells without transfection were used as a control. DAPI was used to stain cell nuclei in blue. Scale bar = 100 μm. (B) Comparison of differentiation capacities of hNSCs on days 20 and 35 ($^{*}P < 0.05$). The positive cells for each marker were counted. At least 6 random fields per sample were captured for quantitative analysis.

group (Fig. 7; n = 3; $P < 0.05$). These results suggest overexpression of Olig1 and Olig2 in hNSCs may inhibit neuronal differentiation of these transfected cells.

Higher levels of myelin basic protein (MBP) were observed in these three transfected groups compared to the control group on day 35 (Fig. 7; n = 3; $P < 0.05$). Specifically, Olig1/2-transfected cells expressed the highest level of both PDGFRα and Nkx2.2 among all the groups (n = 3; $P < 0.05$), which further validates that the combination of Olig1 and Olig2 can more efficiently promote oligodendrocyte differentiation than a single factor.

Schematic summary of our approach on enhancing oligodendrocyte differentiation has shown in Fig. 8. Using human fetal tissue-derived NSCs as a model, we have demonstrated that this non-viral, nanoparticle-mediated transcription activation approach is more effective in generating O4+ oligodendrocytes than a standard differentiation medium (44% *vs.* 5% in a 35-day differentiation culture). In addition, Franklin and colleagues have recently reported a high yield of oligodendrocytes and an acceleration of the overall differentiation program from hESCs at low and physiological (3%) oxygen levels, a mimic environment of the developing forebrain [28]. Culture our Olig1/2-transfected cells in this hypoxia condition may potentially further enhance the yield of O4+ cells. Furthermore, PDGFRα+ OPCs, isolated by fluorescence-activated cell sorting (FACS) using PDGFRα as a marker, have shown robust remyelination following their transplantation in the hypomyelinated shiverer mouse brain [29,30]. In contrast, O4+ OPCs, which could be purified by FACS using O4 as a marker, not only have the comparable remyelination potential, but eliminate contaminant cells and minimize the tumorigenic potential following their transplantation [31]. FACS-purified O4+ cells also hold great potential for *in vitro* myelination assays and screen for myelinating compounds [32–34], thus making our approach a more clinically relevant option.

Given that previous study showed efficient remyelination of transplanted OPCs in a shiverer mice [29], this model could be used for assessing the myelinating ability of nanoparticle-mediated Olig1/2-transfected hNSCs following transplantation. Our nanoparticle transfection approach may also be applied to deliver Olig

genes to engineer endogenous glial cells at the lesion site for the treatment of brain injuries and diseases.

Beyond neural repair in brain tissue, Olig overexpression holds promise in improving spinal cord repair as well. Kim et al. have recently shown that injection of retroviruses encoding Olig1 and Olig2 in combination into the lesion site after contusive spinal cord injury increased the number of endogenous glial progenitor cells without tumor formation and enhanced specification and maturation of oligodendrocytes, leading to improved locomotor recovery. In contrast, Olig1 alone exhibited only modest effects and Olig2 induced the glioma formation in the injured spinal cord [35]. Compared to this retroviruses transduction method, our PBAE-DNA nanoparticle-mediated transfection may offer a safer alternative for *in situ* transcription modification to promote neural repair.

3. Conclusion

We demonstrated the effectiveness of PBAE-DNA nanoparticle-mediated overexpression of Olig1 and Olig2 in enhancing oligodendrocyte differentiation from hNSCs. Our approach can generate around 44% O4+ cells by Olig1/2-cotransfected cells in 35 days. This method provides a translatable approach to modulate oligodendrocyte differentiation and maturation, and has potential to improve the therapeutic outcomes of stem cell-based therapy.

4. Materials and methods

4.1. Materials

Homo sapiens oligodendrocyte transcription factor 2 (Olig2, RG208209) and oligodendrocyte transcription factor 1 (Olig1, SC123482) were purchased from OriGene Technologies (Rockville, MD). Recombinant human fibroblast growth factor-2 (FGF-2), epidermal growth factor (EGF), platelet derived growth factor-AA (PDGF-AA), and neurotrophin 3 (NT-3) were obtained from Millipore (Billerica, MA). Poly (β-amino ester)s (PBAEs) were synthesized as we reported before (Fig. S1) [21,22].

4.2. Cell culture

Human neural stem/progenitor cells (hNSCs), immortalized cells derived from ventral mesencephalon region of fetal brain tissue, were purchased from Millipore (Billerica, MA). Human NSCs were cultured in ReNcell Neural Stem Cell Medium (Millipore, Billerica, MA) with FGF-2 (20 ng/mL) and EGF (20 ng/mL) and used before Passage 5 in this study. In consideration of oligodendrocyte differentiation, hNSCs were cultured in Oligodendrocyte Enrichment Medium [OEM: DMEM/F12 with non-essential amino acid (1×), L-glutamine (2 mM), N21 medium supplement (1×), and a cocktail of growth factors (PDGF-AA, NT-3, and FGF-2; each at 20 ng/mL)] for 17 days. Then cells were continuously cultured in the ReNcell Neural Stem Cell Medium for 14 days.

4.3. Cell transfection

PBAE structures (447, 456, and 536), polymer/plasmid DNA ratios (20, 30, and 40 w/w), and DNA doses (0.3, 0.6, and 1 μg/cm^2) were screened to identify an optimized nanoparticle composition with high transfection efficiency and low cytotoxicity through GFP-tagged Olig2 plasmid DNA. The transfection protocol was followed as we previously reported [21,22]. Olig2-GFP expression efficiencies were measured at days 2 and 9 after transfection, respectively, by a flow cytometry (Accuri C6, BD Biosciences, San Jose, CA) with Hypercyt high-throughput robotic sampler (Intellicyt, Albuquerque, NM). Cytotoxicities of nanoparticles were investigated by WST-1 assay at days 1 and 9 following cell transfection, respectively.

In addition, AlamarBlue assay (Invitrogen, Carlsbad, CA) was performed to examine transfected cell viability in a long-term culture [22]. In order to improve transgene expression, the optimized nanoparticle condition was selected for multiple transfections. The second, third, and forth transfections with the same protocol were conducted at days 13, 20, and 27, respectively.

4.4. Cell differentiation

RNA Isolation and Quantitative Real-Time PCR. The differentiation of hNSCs after transfection with nanoparticles containing plasmid encoding Olig1, plasmid encoding Olig2, or the mixture of these two plasmids (Olig1/2) was examined by real-time PCR. RNA was isolated by the RNeasy Mini Kit (QIAGEN, Frederick, MD). Quantitative RNA was converted to single-stranded cDNA by a High Capacity cDNA Reverse Transcription Kit (Life Techonology, Carlsbad, CA). Primers used for real-time PCR were shown in Table S1.

Immunocytochemistry. The cells were fixed with 4% (w/w) paraformaldehyde at days 20 and 35, respectively. Cells were stained with primary antibodies, including Olig1, Olig2, Tuj1, GFAP, A2B5, PDGFRα, and O4 (Table S2) and then Cy3-conjugated affinity-purified secondary antibodies (Jackson ImmunoResearch, West Grove, PA). Cell nuclei were stained with 4′,6-diamidino-2-phenylindole, dihydrochloride (DAPI, Molecular Probes, Eugene, OR). Then cell images were captured by Zeiss LSM 510 Meta Confocal Microscope (Thornwood, NY). At least 6 random fields per sample were taken for quantitative analysis. The percentage of positive cells in the population was calculated and compared among groups.

Western Blot. Western blot was performed as we previously reported [21]. Protein extracts were quantitated by BCA assay

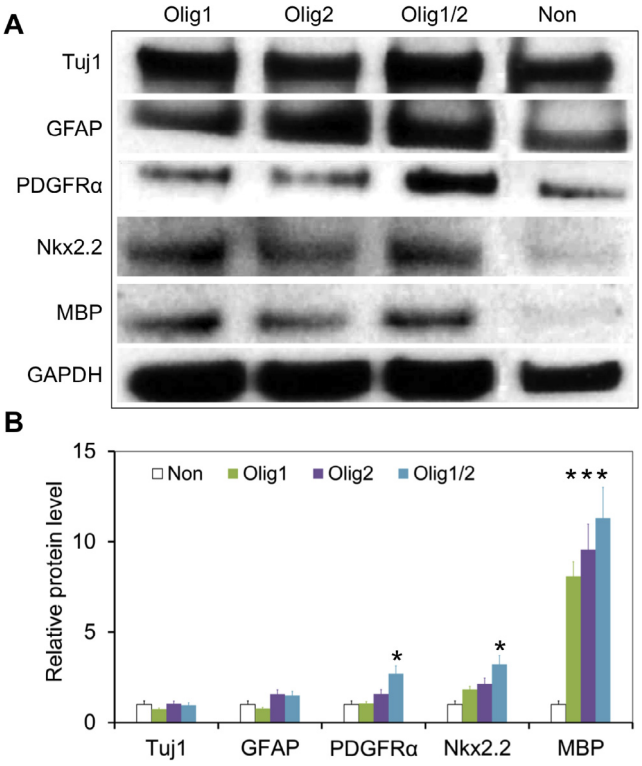

Fig. 7. Western blot analysis of differentiation of transfected hNSCs on day 35. Cells without transfection were used as a control. Olig1/2-transfected hNSCs expressed the highest level of PDGFRα and Nkx2.2 among all the groups on day 35 (n = 3; $^*P < 0.05$).

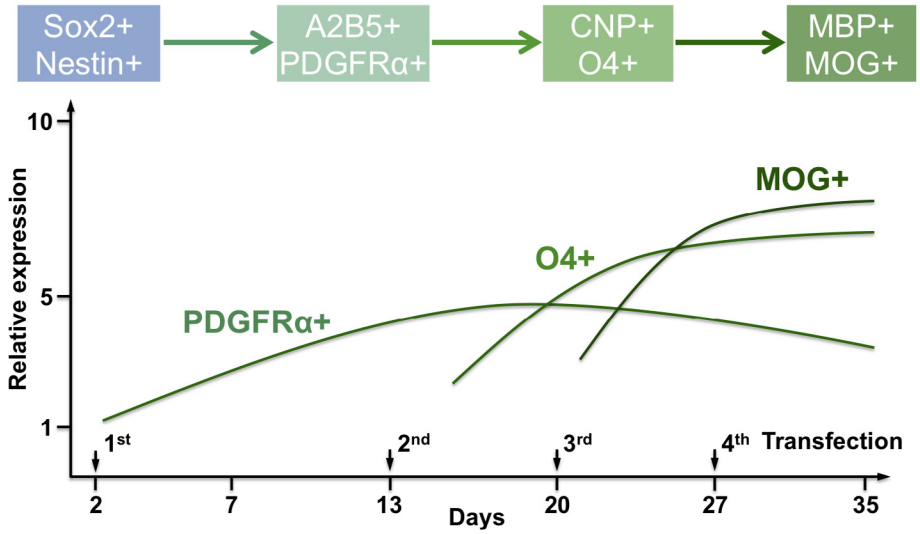

Fig. 8. Schematic summary of enhancing oligodendrocyte differentiation by nanoparticle-mediated Olig expression.

and blotted with antibodies including Tuj1, GFAP, PDGFRα, Nkx2.2, MBP, and GAPDH.

4.5. Statistical analysis

Data were shown as mean ± S.D. (standard deviation). Data were analyzed by Student's *t*-test or one-way ANOVA (analysis of variance) followed by Tukey's post hoc test as needed. The values were considered significantly different at $P < 0.05$.

Author contributions

X.L. contributed to the design, execution, and analysis of the described experiments. S.T. contributed to the polymer synthesis and nanoparticle screening. C.G.Z. contributed to the transfection screening studies. J.G. contributed to the discussion and analysis of transfection studies. V.K. and G.M. contributed to the discussion of the experimental design and interpretation of results. H.-Q.M. conceived the project and contributed to study design and result analysis. X.L. and H.-Q.M. prepared the manuscript with inputs from all authors.

Competing financial interests

The authors affirm no competing financial interests.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actbio.2017.03.032.

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