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Generation of oligodendrocyte precursor-like cells from mouse embryonic fibroblasts using miR-7a mimics

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ABSTRACT

Objective(s): Developing effective strategies to enhance remyelination remains a major therapeutic challenge in demyelinating disorders such as multiple sclerosis (MS). MicroRNAs (miRNAs) have emerged as powerful regulators of cell fate. It has been shown that miR-7a is highly expressed in oligodendrocyte precursor cells (OPCs). This study aimed to investigate whether overexpression of miR-7a can reprogram mouse embryonic fibroblasts (MEFs) into OPC-like cells and oligodendrocytes *in vitro*.

Materials and Methods: MEFs were cultured, confirmed by vimentin immunostaining, and transfected with miR-7a mimics using Lipofectamine™ 3000 or Lipofectamine™ RNAiMAX reagent. Transfection efficiency was assessed by qRT-PCR analysis of miR-7a expression levels. Cells were then cultured in OPC induction medium and assessed for NG2 expression at 14 and 21 days post-transfection. Differentiation potential was examined by transferring induced cells into oligodendrocyte differentiation medium and analyzing O4 and MBP expression using Immunocytofluorescence.

Results: Lipofectamine™ RNAiMAX-mediated transfection produced significantly higher miR-7a expression than Lipofectamine™ 3000, with a clear dose-dependent effect. MEFs transfected with miR-7a (10 and 20 pmol) exhibited NG2-positive cells at both 14 and 21 days, whereas no OPC markers were observed in negative controls or induction medium alone. Furthermore, miR-7a-induced OPC-like cells differentiated into $O4^+$ and MBP^+ oligodendrocytes, confirming their lineage potential.

Conclusion: Overexpression of miR-7a successfully reprogrammed MEFs into OPC-like cells and promoted their differentiation into oligodendrocytes *in vitro*. These findings establish miR-7a as a potential tool for cell-based reprogramming strategies to generate OPC-like cells, warranting further investigation before translation into *in vivo* remyelination procedures for MS.

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Introduction

Cellular reprogramming has emerged as a transformative strategy for generating lineage-specific cells from somatic sources, offering new avenues for disease modeling and regenerative medicine. Direct reprogramming, which bypasses a pluripotent intermediate, has attracted particular attention for its relative efficiency, reduced tumorigenic risk, and preservation of lineage stability (1-3).

In demyelinating diseases such as MS, the failure of endogenous remyelination contributes to progressive neurological disability. Recently, cell-based therapies have been considered novel strategies for repairing demyelinated lesions in MS and related disorders (4). Transplantation of myelin-forming cells, such as oligodendrocyte precursor cells (OPCs), neural stem cells (NSCs), or induced pluripotent stem cell (iPSC)-derived oligodendrocytes, has been shown to replace myelin loss and restore neural function (5). However, challenges such as limited cell availability, inefficient differentiation, ethical concerns, and the risk of tumorigenicity have delayed their clinical application. To overcome these limitations, direct reprogramming, the conversion of one somatic cell type directly into another without passing through a pluripotent stage, has attracted attention. This approach offers a safer and more efficient alternative for generating lineage-specific cells, such as OPC-like cells, from available sources, including fibroblasts (1, 6).

MicroRNAs (miRNAs) are potent post-transcriptional regulators that orchestrate complex gene networks involved in cell fate decisions (7). Through base-pairing with complementary sequences in target mRNAs, miRNAs cause mRNA degradation or prevent translation, therefore modulating extensive cellular processes (8). Since miRNAs regulate the expression of molecules involved in many aspects of normal development and homeostasis, dysregulated miRNA expression has been strongly associated with various clinical disorders. Their main

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role in modulating both physiological and pathological pathways has highlighted miRNAs not only as potential agents in therapeutic applications but also as diagnostic and prognostic biomarkers in clinical practice (8, 9).

Among these, microRNA-7 (miR-7) is a highly conserved miRNA that exhibits regulated spatiotemporal expression from development through adulthood (10). In both humans and mice, mature miR-7 is produced from three distinct genomic loci, highlighting both functional redundancy and the importance of miR-7 in controlling critical cellular pathways (11, 12).

Previous studies have shown that miR-7 is highly expressed in OPCs (13), suggesting a potential role in glial lineage specification. Therefore, this study aimed to evaluate whether overexpression of miR-7 could reprogram mouse embryonic fibroblasts (MEFs) into OPC-like cells *in vitro*. By exploring fibroblasts' capacity for lineage conversion and subsequent differentiation toward oligodendrocytes, we aimed to investigate the potential of miR-7 to promote remyelination.

Materials and Methods

Mouse embryonic fibroblasts (MEFs) culture

MEFs were obtained as a gift from Prof. Simonetta Sipione's lab (Department of Pharmacology, University of Alberta, Edmonton, Canada).

Culture flasks (25 cm^2) were pre-coated with 0.1% gelatin before seeding. Frozen vials of MEFs were thawed and cultured in MEF medium consisting of high-glucose DMEM supplemented with 15% fetal bovine serum (FBS), 2 mM L-glutamine, and 1 mM sodium pyruvate. Cultures were maintained at 37 °C in a humidified incubator with 5% CO_2.

To characterize the MEFs, vimentin immunocytochemistry was performed. MEFs were passaged two to three times, and cells from passages 4–5 were used for all downstream assays (14, 15).

Transfection of MEFs with miR-7a mimics

To evaluate transfection efficiency, MEFs were seeded at an appropriate density in 6-well plates and transfected with either miR-7a mimic or a negative control mimic. Two transfection reagents were compared: Lipofectamine™ 3000 and Lipofectamine™ RNAiMAX (Thermo Fisher Scientific).

Transfections were performed according to the manufacturer's instructions. Briefly, miRNA mimics were diluted in serum-free Opti-MEM and incubated with either Lipofectamine™ 3000 or LipofectamineTM RNAiMAX for 20 min at room temperature to form complexes. The complexes were then added to cells and incubated overnight. The transfection medium was replaced with fresh MEF culture medium. Cells were harvested 5 days post-transfection for RNA extraction and quantitative PCR (qRT-PCR) analysis (15, 16).

RNA extraction and qRT-PCR

Total RNA, including small RNAs, was extracted using the Qiagen miRNeasy kit, following the manufacturer's instructions. RNA concentration and purity were determined using a NanoDrop spectrophotometer.

For reverse transcription, 10 ng of RNA per sample was processed with the TaqMan MicroRNA Reverse Transcription Kit (Applied Biosystems) using primers specific to miR-7a. qRT-PCR was carried out using the TaqMan Universal PCR Master Mix and the TaqMan MicroRNA Assay for miR-7a. U6 small nuclear RNA was used as the endogenous control. Relative expression was calculated using the $2^{-\Delta\Delta Ct}$ method. All reactions were performed in triplicate (15, 17).

Induction of OPC-like phenotype in MEFs

To test whether miR-7a overexpression could reprogram MEFs toward an OPC-like fate, cells at passages 4–5 were seeded onto poly-L-ornithine/laminin-coated coverslips in 24-well plates. The following day, cells were transfected with miR-7a mimic or negative control using LipofectamineTM RNAiMAX. Three days post-transfection, the medium was switched to an OPC induction medium consisting of DMEM/F12 and Neurobasal medium (1:1), supplemented with N2, B27, penicillin/streptomycin, PDGF-AA (10 ng/mL), and FGF2 (10 ng/mL). The induction medium was replaced every other day. Cells were maintained under these conditions for 14 or 21 days. At each endpoint, cultures were fixed for immunocytochemistry. In parallel, primary OPCs isolated from neonatal mouse cortex were cultured as a positive control (18).

Differentiation of induced OPCs into oligodendrocytes

To assess the differentiation potential of induced OPC-like cells, MEFs were transfected with miR-7a mimics at concentrations of 20, 40, or 80 pmol. After 14 days in OPC induction medium, cells were transferred into oligodendrocyte differentiation medium for 5 days. The differentiation medium consisted of DMEM/F12 supplemented with N2, B27, penicillin/streptomycin, triiodothyronine (T3), retinoic acid, and FGF2 (10 ng/mL). The medium was refreshed every other day. At the end of this differentiation period, cultures were fixed for immunocytofluorescence. Cells grown on coverslips were fixed with 4% paraformaldehyde for 10–15 min at room temperature, washed three times with phosphate-buffered saline (PBS), and, when required, permeabilized with 0.3% Triton X-100 for 20 min (for intracellular markers). Non-specific binding was blocked using 10% normal goat serum (NGS) for 1 *hr* at room temperature. Cells were then incubated overnight at 4 °C with primary antibodies against O4 (mouse anti-O4, 1:50) or MBP (rabbit anti-MBP, 1:100). After washing with PBS (three times for 5 min), appropriate fluorophore-conjugated secondary antibodies were applied for 30 min at room temperature in the dark. Nuclei were counterstained with DAPI, and coverslips were mounted using antifade mounting medium. Immunofluorescence images were acquired using a fluorescence microscope (Leica), and all experiments were performed in triplicate (18, 19).

Immunofluorescence staining

Cells cultured on gelatin-coated coverslips were fixed with pre-cold 4% paraformaldehyde for 10–15 min at room temperature and washed three times with PBS. Blocking and permeabilization were performed in PBS with 10% normal goat serum (NGS) and 0.3% Triton X-100 for 1 hour. Samples were incubated overnight at 4 °C with the following primary antibodies: rabbit anti-NG2 (1:250), mouse anti-vimentin (1:500), mouse anti-O4 (1:50), and rabbit anti-MBP (1:100). The next day, after washing with PBS, coverslips were incubated for 30 min at room temperature

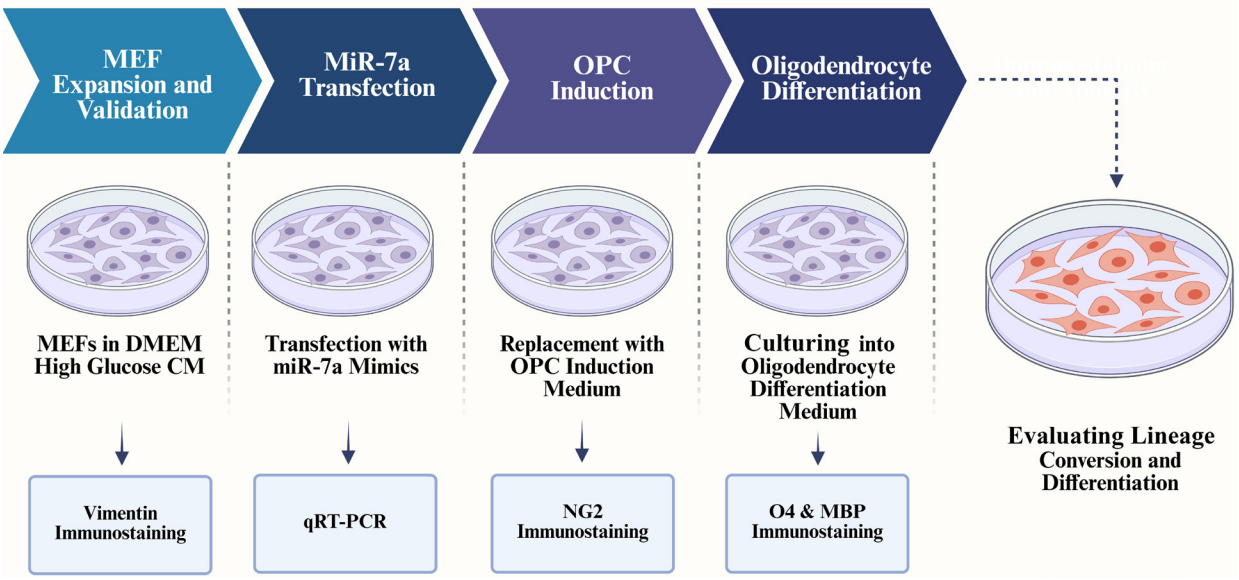

Figure 1. Schematic representation of the experimental design and timeline for miR-7a–mediated reprogramming of MEFs into OPC-like cells
The figure summarizes the stepwise procedure used in the study. Initially, mouse embryonic fibroblasts (MEFs) were expanded and validated in high-glucose Dulbecco's Modified Eagle's Medium (DMEM) and characterized by vimentin immunostaining. Cells were then transfected with miR-7a mimic overnight, followed by quantitative real-time polymerase chain reaction (qRT-PCR) to confirm miR-7a expression. After 3 days, the MEF culture medium was replaced with OPC induction medium, and cells were maintained for 14–21 days, during which NG2 expression indicated the emergence of oligodendrocyte precursor cells (OPCs)-like phenotypes. Subsequently, cultures were transferred into oligodendrocyte differentiation medium to promote maturation, assessed by O4 and myelin basic protein (MBP) immunostaining.

with goat anti mouse cy3 (Jakson ImmumoResearch) or Alexa Fluor 488-conjugated secondary antibodies (1:1000). Nuclei were counterstained with DAPI for 5 min. Coverslips were mounted with antifade mounting medium and sealed with clear nail polish. Imaging was performed on a Leica fluorescence microscope, and all experiments were independently repeated at least three times (18, 19). Figure 1 shows a schematic of the experiments.

Results

Characterization of cultured MEFs

To confirm the identity and purity of cultured mouse MEFs, immunocytochemistry was performed using vimentin, a canonical fibroblast marker. As shown in Figure 2, cultured cells mostly expressed vimentin and presented characteristic fibroblast morphology. On the other hand, no fluorescent background was detected in the negative control group incubated with the secondary antibody, indicating signal specificity.

Evaluation of miR-7a transfection efficiency

To examine the efficiency of miR-7a delivery using two lipid-based reagents: LipofectamineTM 3000 and Lipofectamine™ RNAiMAX. Five days post-transfection, qRT-PCR revealed a significant up-regulation of miR-7a expression in cells transfected with the miR-7a mimic compared with the negative control (P<0.05) (Figure 2). It is also worth noting that LipofectamineTM RNAiMAX resulted in a substantially greater increase in miR-7a levels than Lipofectamine™ 3000.

Regarding further dose-dependency assessment, MEFs were transfected with 10 or 20 pmol of miR-7a mimic using Lipofectamine™ RNAiMAX. As shown in Figure 3, cells transfected with 10 pmol exhibited a significant increase in miR-7a expression compared with negative controls (P =0.0004). Transfection of MEFs with miR-7a mimics at a concentration of 20 pmol resulted in even greater induction of miR-7a, both relative to the negative control (P<0.0001)

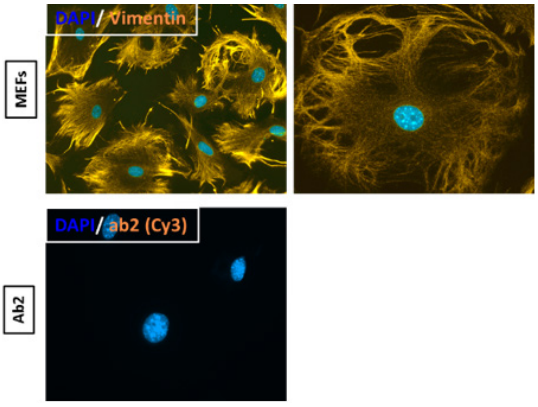

Figure 2. Immunocytofluorescence against vimentin in cultured MEFs
Representative immunofluorescence images showing mouse embryonic fibroblasts (MEFs) stained for vimentin (Cy3, gold) and nuclei counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (blue). Nearly all cultured cells expressed vimentin, consistent with fibroblast identity, whereas no background signal was observed in negative controls incubated with secondary antibody alone. Magnification: 400×.

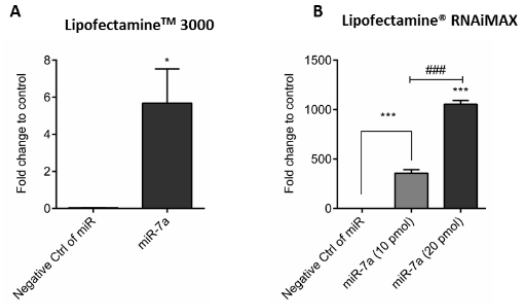

Figure 3. Expression of miR-7a following transfection with Lipofectamine™ 3000 or Lipofectamine™ RNAiMAX
A) Relative expression of miR-7a in MEFs transfected with miR-7a mimic using Lipofectamine™ 3000, measured by qRT-PCR at 5 days post-transfection. B) Dose-dependent induction of miR-7a expression using Lipofectamine™ RNAiMAX. qRT-PCR analysis of mouse embryonic fibroblasts (MEFs) transfected with 10 or 20 pmol miR-7a mimic using Lipofectamine™ RNAiMAX, measured at 5 days post-transfection. Data are shown as mean±SEM. *P<0.05 vs negative control mimic; ***P<0.001 vs negative control mimic; ###P<0.001 vs 10 pmol miR-7a.

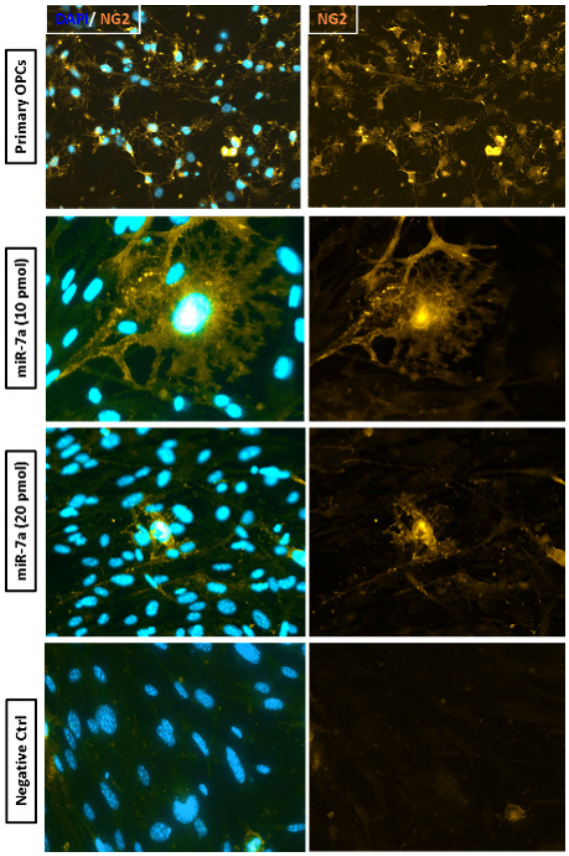

Figure 4. Immunocytofluorescence sections of Direct reprogramming of MEFs into induced oligodendrocyte precursor cells (iOPC) by miR-7a overexpression at day 14

Immunofluorescence staining of primary OPCs isolated from neonatal mouse cortex shows NG2 expression. Mouse embryonic fibroblasts (MEFs) were transfected with miR-7a mimic (10 or 20 pmol) or negative control and cultured in OPC induction medium for 14 days. Immunofluorescence staining for NG2 revealed NG2-positive cells in both 10 pM and 20 pM groups, but not in the negative control. 4′,6-diamidino-2-phenylindole (DAPI) staining (blue) was used to label cell nuclei in all experimental groups. Magnification: 400×.

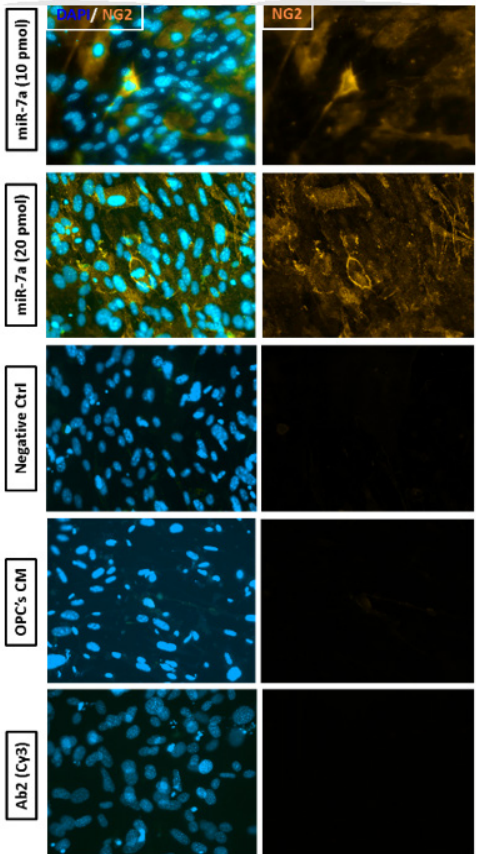

Figure 5. Immunocytofluorescence against NG2 in MEFs at day 21

Mouse embryonic fibroblasts (MEFs) transfected with miR-7a mimic (10 or 20 pmol) and cultured in oligodendrocyte precursor cells (OPC) induction medium for 21 days expressed NG2. No NG2-positive cells were detected in the negative control or induction medium-only groups. 4′,6-diamidino-2-phenylindole (DAPI) staining (blue) was used to label cell nuclei in all experimental groups. Magnification: 400×.

and to the 10 pmol group (P<0.0001). These findings specify Lipofectamine™ RNAiMAX as a more effective reagent and confirm that miR-7a expression in MEFs can be regulated in a dose-dependent manner.

Assess the induction of OPC-like cells by miR-7a overexpression

To determine whether miR-7a overexpression could reprogram MEFs toward an OPC-like fate, MEFs were transfected with a miR-7a mimic and cultured in OPC induction medium. As a reference, primary OPCs isolated from the neonatal mouse cortex were stained with the OPC marker NG2 and showed strong NG2 immunoreactivity. After 14 days, MEFs transfected with 10 or 20 pmol miR-7a exhibited NG2-positive cells, whereas no NG2-positive cells were detected in negative controls or in MEFs exposed only to induction medium (Figure 4).

By day 21, NG2 expression persisted in both 10 and 20 pmol groups, whereas control groups remained negative (Figure 5). Notably, incubation with the secondary antibody alone produced no detectable background, further confirming the specificity of the immunostaining. These results suggest that miR-7a overexpression is likely associated with reprogramming MEFs into OPC-like cells.

Differentiation potential of induced OPC-like cells

We next investigated whether miR-7a-induced OPC-like cells could further differentiate into mature oligodendrocytes. MEFs were transfected with miR-7a at 20, 40, or 80 pmol, maintained in OPC induction medium for 14 days, and then cultured in oligodendrocyte differentiation medium for 5 days.

Immunocytochemical analysis demonstrated that cells in all treatment groups stained positively for O4, a marker of immature oligodendrocytes (Figure 6). O4-positive cells displayed characteristic multipolar or branched morphologies, with extensive cytoplasmic processes extending from the soma. The intensity and number of O4-positive cells appeared to increase with higher miR-7a concentrations, suggesting a dose-dependent enhancement of oligodendroglia. Notably, cells treated with 40 and 80 pmol of miR-7a exhibited more complex process extensions than those treated with 20 pmol, indicating a more advanced stage of differentiation.

To further assess maturation potential, MBP expression, a marker of myelinating oligodendrocytes, was examined (Figure 7). MBP immunoreactivity was detected in cells from all miR-7a-treated groups, confirming their capacity to differentiate beyond the precursor stage. MBP-positive cells showed fine, filamentous staining throughout their processes, resembling the morphology of mature oligodendrocytes in culture.

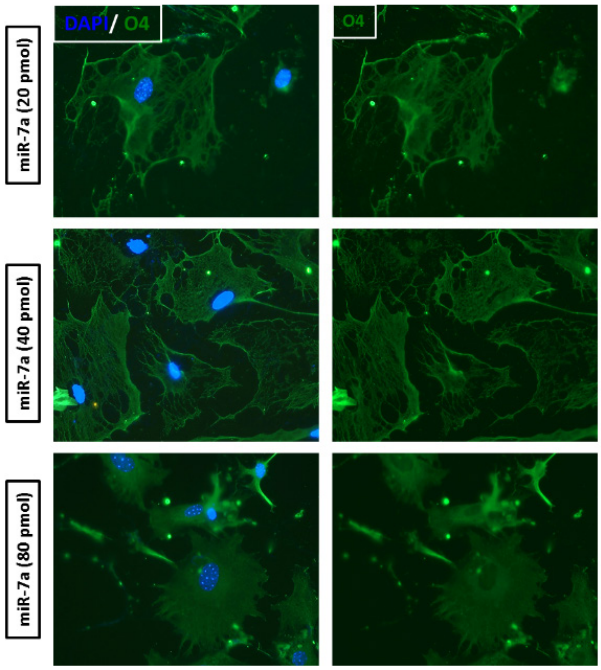

Figure 6. Immunocytofluorescence against O4 in miR-7a-induced oligodendrocyte precursor cells (OPC) in differentiation medium
Mouse embryonic fibroblasts (MEFs) transfected with miR-7a mimic (20, 40, or 80 pmol) were maintained in OPC induction medium for 14 days and then cultured in oligodendrocyte differentiation medium for 5 days. Immunofluorescence staining showed O4 expression in a subset of induced cells, indicating differentiation into immature oligodendrocytes. 4′,6-diamidino-2-phenylindole (DAPI) staining (blue) was used to label cell nuclei in all experimental groups. Magnification: 400×.

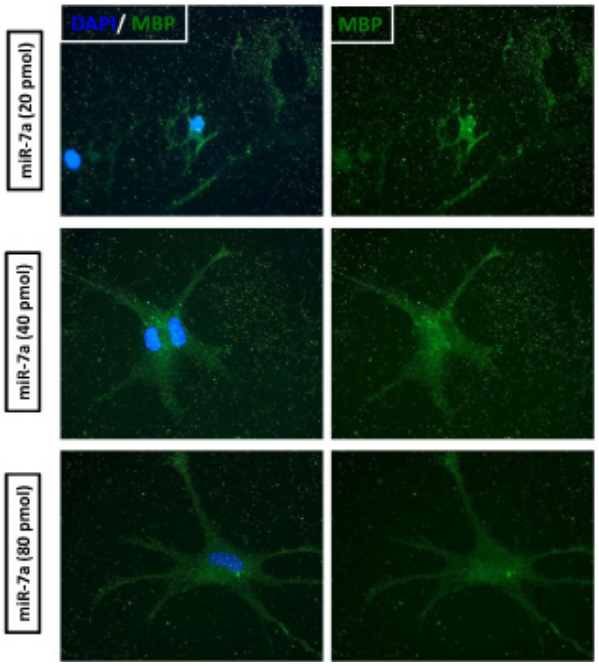

Figure 7. Immunocytofluorescence against MBP in miR-7a-induced oligodendrocytes
Immunofluorescence staining for myelin basic protein (MBP) in mouse embryonic fibroblasts (MEFs) transfected with miR-7a mimic (20, 40, or 80 pmol) and differentiated for 5 days following 14 days in oligodendrocyte precursor cells (OPC) medium. Some induced cells expressed MBP, consistent with maturation into oligodendrocytes. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI) (blue). Magnification: 400×.

Discussion

This study highlights a critical gap in regenerative neuroscience by demonstrating that a single microRNA, miR-7a, can initiate fibroblast-to-oligodendrocyte lineage reprogramming *in vitro*. This finding is notable given that most previously reported strategies for oligodendrocyte generation rely on combinations of transcription factors or complex small-molecule cocktails.

In the present study, MEFs were successfully characterized by vimentin expression, confirming fibroblast characteristics. miR-7a transfection efficiency was evaluated by qRT-PCR, with RNAiMAX showing more viable performance compared to Lipofectamine™ 3000. miR-7a overexpression induced NG2-positive OPC-like cells at both 14 and 21 days, which further differentiated into O4- and MBP-positive oligodendrocytes under the explained differentiation conditions. These results show that miR-7a can initiate lineage reprogramming of MEFs into oligodendrocytes.

In the context of MS, remyelination is considered a critical factor due to key mechanisms, such as axon preservation and restoration of conduction, which, in turn, lead to improved outcomes (20). Fibroblasts are represented as a cellular source due to their accessibility, abundance, and autologous potential. Therefore, the ability to convert fibroblasts into OPC-like cells using a single miRNA provides a valuable pathway for future regenerative designs (21). On the other hand, the efficacy of OPC induction and subsequent differentiation is biologically significant and shows functional progression as a potent agent in applicable therapies (22).

miRNAs exert powerful control over cell fate decisions by simultaneously modulating multiple signaling pathways (23), and miR-7a is no exception, acting as a multi-target regulatory molecule that influences pathways that determine cell fate (24). Mechanistically, miR-7a has been reported to target signaling components such as EGFR, IRS-2, and elements of the PI3K/AKT and MAPK pathways, thereby influencing proliferation, differentiation, and survival programs that are critical for glial development (25, 26). In our study, overexpression of miR-7a in MEFs was sufficient to activate the OPC marker NG2 and drive subsequent expression of O4 and MBP, markers of immature oligodendrocytes, respectively. These findings suggest that miR-7a can override fibroblast identity and redirect lineage specification by reprogramming intracellular signaling and transcriptional networks, like how other miRNAs (e.g., miR-9/miR-124) reprogram fibroblasts into neurons (27). Taken together, our data highlight miR-7a as a regulator of glial lineage reprogramming and suggest its potential as a future cell-based therapeutic strategy, pending viability in *in vivo* models. We hypothesize that miR-7a may facilitate reprogramming by suppressing fibroblast identity genes while promoting OPC-associated transcriptional programs, therefore leading to lineage plasticity.

These results align with the general concept that fibroblasts can be reprogrammed into neural lineage cells under defined molecular cues, as previously demonstrated using transcription factors such as Sox10, Olig2, and Nkx6.2 (28, 29). In line with these findings, a study showed that Sox10 overexpression alone could convert astrocytes into oligodendrocyte-like cells in both *in vitro* and *in vivo* settings, confirming the central role of this transcription factor in glial fate determination (30). Similarly, another investigation showed that Sox2 could induce astrocytes to oligodendrocyte lineage cells in demyelinated mouse brains,

highlighting transcription factor–based reprogramming as an efficient route for remyelination (31).

Additionally, iPSC-based protocols can yield OPCs (32), but these approaches are often time-consuming, involve multiple differentiation steps, and carry pluripotency-associated risks, including tumorigenicity. Small-molecule-based methods have also been reported to facilitate glial induction, but typically require carefully tuned combinations of growth factors and signaling inhibitors to achieve efficiency (33). For instance, one study used the histone deacetylase inhibitor Trichostatin A (TSA) to promote the conversion of astrocytes into OPCs (34). At the same time, another study showed that small molecules, including CHIR99021, Forskolin, and valproate, could induce astrocyte-to-OPC conversion both *in vitro* and after transplantation into demyelinated mice (35). These chemical-based studies highlight the feasibility of non-genetic manipulation to generate OPCs and further position our miR-7a-mediated reprogramming as a molecularly targeted yet simplified approach.

In contrast, our findings demonstrate that a single microRNA, miR-7a, is sufficient to initiate fibroblast-to-OPC reprogramming and promote subsequent differentiation into oligodendrocytes *in vitro*. This is particularly notable because previous studies have often required multiple transcription factors or small molecules to achieve similar lineage outcomes. The use of miR-7a offers several potential advantages: it avoids genomic integration, is more straightforward to deliver (e.g., via synthetic mimics or nanoparticles), and may provide a safer and more clinically appropriate approach compared to gene-based methods. While the efficiency of reprogramming remains to be optimized, these results highlight miR-7a as a promising strategy for a simplified, patient-specific therapy for demyelinating diseases.

Our previous comparable work reported that the miR-302/367 cluster, together with valproate, could convert astrocytes into oligodendrocyte progenitor and myelinating cells *in vivo*, resulting in improved behavioral and histological recovery in demyelinated mice (36). Likewise, another study showed that OCT4 expression, in combination with valproic acid, enhanced myelin repair in a lysolecithin-induced demyelination model (37). These studies show that both transcription factor- and miRNA-based strategies can promote glial reprogramming and remyelination.

Taken together, this evidence significantly elevates expectations regarding miR-7a's potential in clinical practice and its therapeutic implications. OPCs, as mentioned before, are necessary for remyelination, and a reliable source of these cells could aid regenerative strategies, especially in the context of MS treatment (38). It is also worth noting that fibroblasts can be easily obtained from patients, such as during skin biopsies, which enables autologous, patient-specific operations that significantly minimize immune-mediated rejection. Thus, future studies should investigate whether miR-7a-based strategies, including direct delivery approaches, can effectively induce reprogramming and remyelination in ongoing *in vivo* models.

While our findings suggest that miR-7a can reprogram MEFs into OPC-like cells capable of differentiating into oligodendrocytes, several directions remain to be explored. Our study demonstrated the efficacy through cultures, and animal model validations are yet to be tested *in vivo*. Also, while markers were expressed, functional tests of myelination capacity *in vivo* are still needed. Hence, *in vivo* validation in experimental models of demyelination (e.g., cuprizone, lysolecithin, or experimental allergic encephalomyelitis (EAE) mice) will be essential to confirm whether miR-7a-induced cells can integrate into the CNS and promote functional remyelination. Due to ethical and logistical constraints, MEFs were obtained for use as an alternative to patient fibroblasts. Therefore, it is obvious that this may not completely replicate human conditions. Repeating these experiments using human fibroblasts will help determine the translatability of our approach and reveal potential species-specific differences in reprogramming efficiency. Eventually, the efficiency of reprogramming can be further quantified and optimized since not all fibroblasts were converted in this paper. Further mechanistic studies are needed to define the downstream targets of miR-7a and the signaling pathways that govern lineage conversion; such insights could enable the rational design of combination strategies with other miRNAs, transcription factors, or small molecules to enhance efficiency and maturation. Finally, translation into therapeutic application will require the development of safe delivery methods for miR-7a, including non-viral nanoparticles, exosomes, or chemical modifications that allow for *in vivo* reprogramming directly within demyelinated lesions. Collectively, these suggested future studies will determine whether miR-7a-based strategies can progress as a regenerative approach for MS and potentially other demyelinating diseases.

Conclusion

In summary, this study demonstrates that miR-7a overexpression can reprogram MEFs into OPCs, which then differentiate into immature ($O4^+$) and mature (MBP^+) oligodendrocytes *in vitro*. These findings highlight miR-7a as a regulator of glial lineage specification and provide a method for generating OPCs. By establishing miRNA-driven fibroblast-to-oligodendrocyte reprogramming, our work advances the development of safe and efficient regenerative strategies to promote remyelination in MS and related demyelinating disorders.

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The schematic figure was created using BioRender.com.

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Data Availability

The data will be made available upon request.

Authors' Contributions

M GK and F G designed the experiments; M GK and M NN conducted the experiments and gathered data; M GK, M NN, F R, and F G analyzed the results and discussed the strategy; M GK and F G supervised, directed, and managed the study; M GK, M NN, F R, and F G approved the final version for publication.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Declaration

We have not used any AI tools or technologies to prepare this manuscript.

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