The Determinant Role of miR-19 in Neural Stem Cell Fate: The Red Light to the Neuronal Differentiation and the Green Light to the Oligodendrocyte Differentiation

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Abstract

Background The precise epigenetic pathways and mechanisms involved in determining the fate of neural progenitor stem cells (NPCs) into differentiated neural lineages including oligodendrocytes (OLs) is an unresolved issue. MiRNAs are one of the key differentiating factors whose aberrant expression has been shown to disturb proper development. In the present study the leading role of miR-19 in NPCs differentiation to OLs was investigated.

Methods MiR-19 was overexpressed in hiPSC derived NPCs and for confirming differentiation to the desired cell line, i.e. OL, the expression of cell line-specific genes was evaluated by Real-Time PCR. Oligodendrocyte progenitor cells (OPCs) precursor in the differentiated cells was studied by Flow cytometry. Luciferase activity was also measured to confirm the interaction of miR-19 with the 3'UTR of mRNA of the putative genes.

Results Our results indicate that the upregulation of miR-19 is a contributing factor in determining NPCs' fate and the timing of progenitor division arrest. MiR-19 can cause a significant expression of OL markers. Besides, miR-19 repressed proneuronal differentiation factors including NeuroD1, NeuroD4, and neurogenin. Moreover, the higher expression of miR-19 could downregulate SOX4, SOX5, and SOX6 genes, which are involved in inhibiting OL differentiation and maturation.

Conclusions Our finding demonstrated the functional link of miR-19 to regulatory networks of NPCs differentiation, as well as introduced a new strategy for modulating OL fate commitment and development of novel cell therapies.

1. Background

Numerous people worldwide suffer from demyelinating diseases. In these situations, the myelin sheath that covers neural axons and facilitates the conduction of neural signals is lost. The most common form of these diseases is multiple sclerosis, MS (1, 2). In the patients of MS, the immune system attacks myelin sheaths and degenerate them. Another common incident in MS is the loss of oligodendrocytes (OLs) which makes myelin formation a very slow process and exacerbates the disease because OLs are involved in protecting neural axons and producing myelin in the central nervous system. Accordingly, an appropriate treatment strategy for this disease could be the replacement of the lost OLs to accelerate the myelin-forming process (3–5).

A viable method for replacing lost or damaged cells is cell Therapy. Cell therapy is based on injecting living cells to make up certain deficient cells. Among different cell therapy approaches, stem cell therapy is the most promising method for treating diseases which require injecting a specific cell in a high number (5–7). Therefore, the main obstacle for stem cell therapy is to produce enough amount of a particular differentiated cell. In order to address this issue, scientists use various stem cell propagation and differentiation methods to produce enough cellular mass of a specific cell in the laboratory

environment. There are different methods for stem cell differentiation, including using small molecules and growth hormones (8, 9).

MicroRNAs are the major non-coding RNA molecules which have a pivotal role in most of the gene regulatory mechanisms at the transcript level (10–12). It appears that miRNAs have the highest diversity of expression in the brain and emerge as important regulators of various aspects of CNS development. They modulate, mainly downregulating, expression of regulatory genes. Their function will change the major active signaling networks in specific cell types and thereby, control fate specification and differentiation of neural stem cells (13–16). Recently, OL development and miRNA fields have converged with the identification of key miRNAs that are required for leading the differentiation pathway of NPCs towards OLs (17–21). NPCs are the remnants of the neural stem cell in the adult brain and are responsible for producing both neuron and glia cells. The NPCs' differentiation towards OLs can be enhanced by blocking the pathway of neuronal differentiation and promoting the pathway of glia cells. A series of evidence indicates that several miRNAs are involved in the cell fate decision of NPCs (22). For instance, miR-124 is highly enriched in the CNS and regulates neural/glial specification. Overexpression of the miR-124 represses expression of many non-neuronal genes and activates a gene expression program similar to that of neuronal cells (23, 24). Interestingly, miR-19 exhibits a low level of expression in human neural progenitor stages, while it is up-regulated during the early oligodendrocyte progenitor cell (OPC) transition. Also, its expression remains high during mid to late OPC differentiation (25). In this context, we speculate the importance of miR-19 in the NPCs' different stages of differentiation. So, in the current study, the role of miR-19 in NPC differentiation toward OPC was investigated. The results indicated that miR-19 overexpression effectively blocked neural fate decisions and promoted the expression of OL differentiation markers through the prevention of the neural-specific gene expression.

2. Materials And Methods

2.1. Cell line culture and differentiation

A human induced pluripotent stem cell (hiPSC) derived NPC line, from Royan Institute, Tehran, Iran, was proliferated on poly-D-lysine (PDL)-coated plates using neurobasal medium (Gibco) supplemented with 20 ng/ml epidermal growth factor (EGF), 1x penicillin/streptomycin, 2 mM L-glutamine, and 25 ng/ml bFGF (All from Invitrogen). For OPC differentiation, NPCs were grown for 3 weeks in the oligo medium containing serum-free DMEM/HAMS F12 medium (Gibco) supplemented with 1% bovine serum albumin, 2mM L-glutamine, 50 µg/ml gentamicin, 1 × N2 supplement, 3 nM T3 (Sigma), 2 ng/mL Shh (Sigma), 2 ng/mL NT-3 (Sigma), 20 ng/mL bFGF, and 10 ng/mL PDGF-AA (Sigma).

Next, OL differentiation from OPCs was triggered by removing growth factors and culturing cells for two additional days.

Culture of human embryonic kidney cells (HEK293T) was done using Dulbecco's modified eagle's medium (DMEM) containing 10% fetal bovine serum (FBS, Hyclone, USA) and 1x penicillin/streptomycin. Cells

were incubated in humid condition, 5% CO₂ at 37° C.

2.2. Lentiviral vector construction and infection

MiR-19 precursor spanning sequence was PCR-amplified with miR-19-1 FW 5'-

TACTCGAGTCTACTGCCCTAAGTGCTCC – 3' and miR-19-1 RV 5'-

TAGACGCGTGCTGTACATCAGATAGACCAG – 3' primers and cloned into the Xho I/ Mlu I restriction site of a mammalian expression vector pLEX.jRED vector. The backbone of the vector (pLEX-Ctrl) and a vector containing scrambled sequence (pLEX-Scr) were used as control vectors. The scrambled sequence was designed using “InvivoGene” servers.

Using the calcium phosphate transfection method, the packaging of the lentiviral particles, containing miR-19 or control sequences, was performed. In this procedure, 10 ug of pLEX-miR-19, pLEX-Ctrl, or pLEX-Scr along with 10 µg of pPAX2 and 5 µg of pMDG plasmids were used. The lentiviral supernatant of transfected HEK293T cells was harvested for three days in 12 hours intervals and concentrated using ultracentrifugation at 25000 rpm for 2.5 h at 4-degree centigrade. The titer of lentivirus particles was determined using flow cytometry analysis of jRED positive HEK293T cells.

2.3. RNA extraction and qRT–PCR

Total RNA was extracted from all transduced and control groups using Trizol reagent (Invitrogen, USA) according to the manufacturer’s protocol on days 7, 14 and 23. cDNA was synthesized from 1 µg of total RNA with M-MLV Reverse Transcriptase (Fermentas, USA) and random primers for mRNA genes, or specific stem-loop RT adaptor primers for miRNA and SNORD47. Real-time PCR was carried out with the reagents of a Sybr green I mix (Takara, China) in a 20 ml reaction volume with specific forward and reverse primers. Data analyses were performed using the $2^{-\Delta\Delta C_t}$ method. Primer sequences are listed in Supplementary Table S1.

2.4. Flow cytometry analysis

Differentiation toward OPC precursor was examined through Flow cytometry (ABI) 14 days after transfection/transduction against OLIG-2, NKX2.2, GFAP, MAP2 and NSE (using mouse monoclonal primary antibodies, from Abcam) and 2 days after the removal of growth factors against MBP on NPCs (using mouse anti-MBP primary antibody, myelin basic protein, from Abcam). Incubation with antibodies was performed at 4° C for at least 30 minutes followed by the secondary antibody treatments (Millipore, Billerica, MA). For cell surface antigens, cells were gently scraped from the culture dishes. To detect antigens that are not present on the cell surface, cells were fixed and permeabilized to disrupt the cell membrane and allow entry of the antibody.

2.5. Luciferase assay

The 3'UTR sites of some of the most important neural differentiation factors, including NeuroD1, NeuroD4, Neurogenin, SOX4, SOX5, and SOX6 were cloned into the pSICHECK2 luciferase reporter vector. HEK cells were co-transfected with the 3'UTR-pSICHECK2 vectors and pLEX-miR-19 or pLEX-Mut-miR-19

(Fig. 4A) in 24-well plates using Lipofectamine 2000 (Invitrogen). The mut-miR-19 construct was generated by the creation of two changes in the seed sequence of miR-19 and cloned in shRNA format into the pLEX.jRED vector. Following 48 hours of incubation, luciferase activity was measured using a dual-luciferase reporter assay system (Promega). The result was calculated by normalizing the Renilla signal to the Firefly signal.

2.6. Database prediction of miRNA targets

The miR-19 targets were predicted using the TargetScan web server (<http://www.targetscan.org>, V.5.1). Early annotation of this target list was performed using the database for annotation, visualization and integrated discovery (DAVID) v6.8.

2.7. Statistical analysis

All experiments were performed at least three times, presented as mean $\pm$ SD and analyzed by student's t-test. p-values of ≤ 0.05 were considered as statistically significant.

3. Results

3.1. NPCs can be efficiently directed to OPC fate via miR-19 up-regulation

To determine whether miR-19 promotes OPC commitment in NPCs, the effects of miR-19 overexpression were examined through the transduction of NPCs with pLEX-miR-19 (Fig. 1A). After 7, 14- and 23-days post-induction of OPC differentiation, specific markers of neuronal cells, astrocytes and OPCs were analyzed by qRT-PCR (Fig. 1B, C, and D). MiR-19 overexpression in the NPCs led to a significant increase in the early markers of glial progenitor cells, OLIG2 and NKX2.2 relative to the controls on day 7, day 14 and day 23. However, MBP expression was only observed on day 23. There were marked decreases in neuronal-specific markers (NeuroD1, NeuroD4, Neurogenin, SOX4, SOX5, and SOX6) on the 23rd day after induction. Moreover, the expression level of LINGO1 and GFAP in transduced cells significantly decreased in this time point. These findings suggest that miR-19 induces OPC development in NPCs.

3.2. MiR-19 enhanced the number of OPC fate cells

Transduced cells were subjected to flow cytometry for stage-specific oligodendrocyte markers (OLIG2, NKX2.2 and MBP), neuronal markers (MAP2 and NSE) and astrocyte marker (GFAP). As shown in Fig. 2, based on the expression of the OL early markers approximately 80% of transduced progenitor cells committed to differentiation into OL cells. Flow cytometry results demonstrated that in the pLEX-miR-19 group, miR-19 overexpression increased the number of the cells expressing NKX2.2 and OLIG2 ($69.8 \pm 2.34\%$ and $82.6 \pm 2.25\%$, respectively, compared with $50.8 \pm 1.06\%$ and $61.09 \pm 2.12\%$ of the pCDH-Ctrl) on the day 14. By contrast, only a few transduced cells expressed non-oligodendrocyte markers. Overexpression of miR-19 resulted in a significant reduction in the number of GFAP expressing cells in

transduced cells ($8.12 \pm 4.15\%$) compared to controls ($27.8 \pm 1.16\%$, $p < 0.05$). Also, the population of MAP2 and NSE positive cells was markedly lower in transduced cells than in the control group. On the day 23, the percentage of positive cells that expressed OLIG2 accounted for $94.6 \pm 1.94\%$, and the percentage of positive cells that expressed Nkx2.2 accounted for $81.5 \pm 2.78\%$ of the cells in the transduced group (compared with $73.4 \pm 2.43\%$ and $62.89 \pm 1.65\%$ of the pCDH-Ctrl, respectively). Transduction of miR-19 increased the percentage of positive cells for MBP up to $\sim 50\%$; about twofold increase compared to the control on day 23, but did not change the percentage of MBP expressing cells on the day 14 (Fig. 2). These findings suggest that miR-19 directs the distinction path of NPCs to the OL lineage.

3.3. MiR-19 promotes oligodendrocyte differentiation through targeting of neuronal key factors

The mechanisms by which miR-19 promotes OL lineage commitment are unknown. To recognize potential physiological targets of miR-19, prediction first was performed with the Target Scan prediction algorithms, and then examined by database for annotation, visualization and integrated discovery (DAVID) v6.8. According to DAVID retrieval, the most frequent target tissue of miR-19 was the brain, by 54.7 percent (Fig. 3A). It confirms that most of the *miR-19* targets have key roles in the brain. A review of the targets expressed in the brain showed that high-score targets are mostly involved in the neuronal differentiation. These targets include NeuroD1, NeuroD4, Neurogenin, SOX4, SOX5 and SOX6, which was predicted to include 4, 1, 1, 2, 2 and 2 sites, respectively (Fig. 3B).

The 3'UTRs of these six genes were cloned into the psiCheck-2 plasmid and co-transfected into HEK cells. The luciferase reporter assay indicated that the activity of the reporter containing the 3'UTR of the target genes was decreased following transfection with pLEX-miR-19, whereas the activity of the reporters co-transfected with pLEX-mut-miR-19 was not altered (Fig. 4B).

Taken together, our results demonstrated that miR-19 through targeting of neuronal differentiation key factors blocked distinction towards the neuronal lineage and paved the way for the differentiation of the oligodendrocyte.

4. Discussion

Transcription factors that are required for neuron lineage direction negatively regulate oligodendrocyte differentiation (25–27). Therefore, suppression of these factors is likely to promote the neuronal to oligodendrocyte fate switch, although the mechanism remains unclear. One of the highly expressed miRNAs in the CNS that regulates neural/glial specification is miR-19. During neural differentiation, miR-19 is undetectable or expressed at low levels. In the current study, bioinformatics results demonstrated that miR-19 has strong evolutionarily conserved target sites on 3'UTR of many key genes in the neurogenesis process including NeuroD1, NeuroD4, Neurogenin, SOX4, SOX5 and SOX6.

Therefore, to address the mechanism of miR-19 action in fate decision, NSCs were transduced with pLEX-miR-19 lentiviruses and evaluated for expression of key markers of neuronal, astrocyte and oligodendrocyte lineages. Our results showed that overexpression of the neurogenic miR-19 in NSCs represses several neuronal genes (NeuroD1, NeuroD4, Neurogenin, SOX4, SOX5 and SOX6) and astrocyte gene (GFAP), while increased OL specific gene markers and function as a promoter to induce oligodendroglial identity.

For the quantitative evaluation of positive cells flow cytometry, instead of the ICC assay, was applied to show the exact number of differentiated cells. The number of differentiated cells toward OPC was about 20% higher in transduced cells than controls.

In this study, luciferase data demonstrates that NeuroD1, NeuroD4, Neurogenin, SOX4, SOX5 and SOX6 that are important for neural differentiation are direct targets of miR-19.

NeuroD1 also called $\beta 2$, expressed in the nervous system late in development and is, therefore, more likely to be involved in terminal differentiation, neuronal maturation and survival of neurons in the adult SGZ and SVZ. In *Xenopus*, overexpression of NeuroD1 converts embryonic epidermal cells into fully differentiated neurons and promotes premature cell-cycle exit and differentiation of neural precursor cells (28–30).

Neurogenin along with E-protein binds to E box elements that are thought to be critical for neural differentiation (27). Neurogenin has a dual function that is partly responsible for the timing of neural differentiation that dominates over glial differentiation. Oligogenesis is marked by neurogenin downregulation allowing progenitor cells to respond to glial-inducing factors. In this way, the temporal control of neurogenin expression may orchestrate the sequential onset of cortical neuronal and glial differentiation (31–33). In fact, in the absence of neurogenin, cortical gliogenesis might commence at an earlier time during development. In support of this, in our studies when pLEX-miR-19 is introduced into the NSCs, it results in blocking neurogenesis and premature gliogenesis through neurogenin downregulation.

NeuroD4 (otherwise known as Math3 and NeuroM) is a member of the bHLH family of transcription factors that is being expressed and phosphorylated during primary neurogenesis and acts downstream of Ngn2 in primary neurogenesis. It is alone sufficient to generate functional neurons, although not sufficient to elicit a neuronal subtype identity (34, 35). In the present study, luciferase assay data confirmed NeuroD4 as one of the miR-19 targets. So, blocking neuronal differentiation at an early stage through suppression of NeuroD4 may be beneficial to the development of oligodendrocyte lineage.

SOX4, as one of the SoxC family members, had been implicated in initiating the early stages of neuronal differentiation both in the adult and embryonic neural progenitors. However, SOX4 overexpression inhibits oligodendrocyte differentiation in mice (36, 37). SOX4 activated transcription of genes associated with neural development in NSCs such as β III-tubulin, MAP2 and doublecortin and reduced the expression of genes promoting oligodendrocyte differentiation. In intermediate neural progenitor cells, SOX4 promotes their maintenance by interacting with Neurogenin2 to activate Tbrain2. On the other hand, SOX4 also

functions through HES5 which is a repressor of oligodendrocyte differentiation and myelination and directly activated by SOX4 (36). Therefore, miR-19 may direct NSC differentiation towards oligodendrocytes by repressing the expression of SOX4, shedding light on a novel regulator of oligodendrocyte differentiation.

SOXD group proteins Sox5 and Sox6 are essential during nervous system development and exist in many different cell types, including VZ progenitors, radial glia, oligodendrocyte precursors, and several neuronal subpopulations (37). In OLs, SoxD proteins repressed myelin gene promoters when bound exclusively. Previous studies indicated that in the absence of Sox5 and Sox6, oligodendrocytes start to differentiate prematurely and at higher numbers. In neurons, SOX5 acts as an important brake on WNT–b-catenin mitogenic activity in neural progenitors and its overexpression leads to premature cell cycle exit and prevents terminal differentiation (38).

SOX6, on one hand, could cause the activation of Wnt-1, Mash-1, N-cadherin, and MAP2 genes, leading to neurogenesis. On the other hand, Sox6 induced cell adhesion molecules, such as E-cadherin or N-cadherin, promoting neuronal differentiation by stimulating cellular aggregation or the cell to cell interaction (39, 40). According to our results, miR-19 could target SOX5 and SOX6 genes and so of these targets it can also play a role in inhibiting the neuronal pathway and promoting OL differentiation.

In summary, our results suggesting that miR-19 has several target genes through which neural differentiation is blocked and OL differentiation is facilitated. Since overexpression of miR-19 may restrict differentiation of NPCs to the oligodendrocyte lineage.

4.1. Conclusions

Present data suggest that miR-19 could regulate NPCs toward OL differentiation through multiple targets that important for fate decision pathways (Fig. 4C).

The current study reveals a new context for potential miRNA-based regulatory applications in the differentiation induction of stem cells. Overexpression of miR-19 is likely to be beneficial during OL lineage permission. Today, exogenous stem cell transplantation in the CNS might carry a therapeutic potential in MS disease. Genetic modification of stem cells for ectopic expression of miR-19 can both shorten the time of differentiation and increases the number of OL differentiated cells.

Abbreviations

3'UTR

3' untranslated region

bFGF

Basic Fibroblast Growth Factor

CNS

Central Nervous System

Ctrl

Control
DAVID
Database For Annotation, Visualization And Integrated Discovery
DMEM
Dulbecco's Modified Eagle's – Medium
EGF
Epidermal Growth Factor
FBS
Fetal Bovine Serum
GFAP
Glial Fibrillary Acidic Protein
HEK293T
Human Embryonic Kidney Cells
hiPSC
Human Induced Pluripotent Stem Cells
ICC
Immunocytochemistry
LINGO1
Leucine Rich Repeat And Ig Domain Containing 1
MAP2
Microtubule Associated Protein 2
MBP
myelin basic protein
miR
MicroRNA
MS
Multiple Sclerosis
NeuroD
Neuronal Differentiation
Ngn2
Neurogenin-2
NPCs
Neural Progenitor Stem Cells
NSCs
Neural stem cells
NSE
Neuron-specific enolase
OLIG2
Oligodendrocyte Transcription Factor 2
OLs

Oligodendrocytes
OPCs
Oligodendrocyte Progenitor Cells
PCR
Polymerase Chain Reaction
PDL
poly-D-lysine
qRT-PCR
Quantitative Reverse Transcription Polymerase Chain Reaction
Scr
Scramble
SD
Standard Deviation
SOX
SRY-Box Transcription Factor

Declarations

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and materials: All data generated or analysed during this study are included in this published article [and its supplementary information files].

Competing interests: The authors declare that they have no competing interests

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Authors' contributions: GSh roles were the project administration, collection, and assembly of data. RR participated in investigation, methodology and analysis. ME provide the study materials. NT is the major contributor in writing the manuscript and analyzing some data. MF analyzed the data and interpretation as well as validation. MH performed data curation and Visualization. Conception and design of the project, as well as administrative support, which was given by FK.

All authors read and approved the final manuscript.

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References

1. Dobson R, Giovannoni G. Multiple sclerosis—a review. *European journal of neurology*. 2019;26(1):27–40.
2. Goldenberg MM. Multiple sclerosis review. *Pharmacy and Therapeutics*. 2012;37(3):175.
3. Lassmann H, van Horssen J. The molecular basis of neurodegeneration in multiple sclerosis. *FEBS letters*. 2011;585(23):3715-23.
4. Eiman MM. Elucidating the Molecular Basis of Multiple Sclerosis and Understanding the Disease Pathophysiology. *Immunome Research*. 2016;12(3):1.
5. Miljković D, Spasojević I. Multiple sclerosis: molecular mechanisms and therapeutic opportunities. *Antioxidants & redox signaling*. 2013;19(18):2286 – 334.
6. Zakrzewski W, Dobrzyński M, Szymonowicz M, Rybak Z. Stem cells: past, present, and future. *Stem cell research & therapy*. 2019;10(1):1–22.
7. Aijaz A, Li M, Smith D, Khong D, LeBlon C, Fenton OS, et al. Biomanufacturing for clinically advanced cell therapies. *Nature biomedical engineering*. 2018;2(6):362 – 76.
8. Shroff G. A review on stem cell therapy for multiple sclerosis: special focus on human embryonic stem cells. *Stem cells and cloning: advances and applications*. 2018;11:1.
9. Cuascut FX, Hutton GJ. Stem cell-based therapies for multiple sclerosis: current perspectives. *Biomedicines*. 2019;7(2):26.
10. O'Brien J, Hayder H, Zayed Y, Peng C. Overview of microRNA biogenesis, mechanisms of actions, and circulation. *Frontiers in endocrinology*. 2018;9:402.
11. Treiber T, Treiber N, Meister G. Regulation of microRNA biogenesis and its crosstalk with other cellular pathways. *Nature reviews Molecular cell biology*. 2019;20(1):5–20.
12. Kouhkan F, Alizadeh S, Kaviani S, Soleimani M, Pourfathollah AA, Amirzadeh N, et al. miR-155 down regulation by LNA inhibitor can reduce cell growth and proliferation in PC12 cell line. *Avicenna journal of medical biotechnology*. 2011;3(2):61.
13. Rajman M, Schratt G. MicroRNAs in neural development: from master regulators to fine-tuners. *Development*. 2017;144(13):2310-22.
14. Díaz NF, Cruz-Reséndiz MS, Flores-Herrera H, García-López G, Molina-Hernández A. MicroRNAs in central nervous system development. *Reviews in the Neurosciences*. 2014;25(5):675 – 86.
15. Tavakolpour V, Shokri G, Moghadasi AN, Nahavandi PM, Hashemi M, Kouhkan F. Increased expression of mir-301a in PBMCs of patients with relapsing-remitting multiple sclerosis is associated with reduced NKRF and PIAS3 expression levels and disease activity. *Journal of neuroimmunology*. 2018;325:79–86.

16. 16. Torabi S, Tamaddon M, Asadolahi M, Shokri G, Tavakoli R, Tasharrofi N, et al. miR-455-5p downregulation promotes inflammation pathways in the relapse phase of relapsing-remitting multiple sclerosis disease. *Immunogenetics*. 2019;71(2):87–95.
17. 17. Afrang N, Tavakoli R, Tasharrofi N, Alian A, Sohi AN, Kabiri M, et al. A critical role for miR-184 in the fate determination of oligodendrocytes. *Stem cell research & therapy*. 2019;10(1):112.
18. 18. Wang E, Cambi F. MicroRNA expression in mouse oligodendrocytes and regulation of proteolipid protein gene expression. *Journal of neuroscience research*. 2012;90(9):1701-12.
19. 19. Letzen BS, Liu C, Thakor NV, Gearhart JD, All AH, Kerr CL. MicroRNA expression profiling of oligodendrocyte differentiation from human embryonic stem cells. *PloS one*. 2010;5(5):e10480.
20. 20. Lau P, Verrier JD, Nielsen JA, Johnson KR, Notterpek L, Hudson LD. Identification of dynamically regulated microRNA and mRNA networks in developing oligodendrocytes. *Journal of Neuroscience*. 2008;28(45):11720-30.
21. 21. Budde H. Role of miRNAs in Oligodendrocyte Development: Niedersächsische Staats-und Universitätsbibliothek Göttingen; 2010.
22. 22. Zhao X, He X, Han X, Yu Y, Ye F, Chen Y, et al. MicroRNA-mediated control of oligodendrocyte differentiation. *Neuron*. 2010;65(5):612 – 26.
23. 23. Sun Y, Luo Z-M, Guo X-M, Su D-F, Liu X. An updated role of microRNA-124 in central nervous system disorders: a review. *Frontiers in cellular neuroscience*. 2015;9:193.
24. 24. Cao X, Pfaff SL, Gage FH. A functional study of miR-124 in the developing neural tube. *Genes & development*. 2007;21(5):531-6.
25. 25. Nielsen JA, Lau P, Maric D, Barker JL, Hudson LD. Integrating microRNA and mRNA expression profiles of neuronal progenitors to identify regulatory networks underlying the onset of cortical neurogenesis. *BMC neuroscience*. 2009;10(1):98.
26. 26. Najm FJ, Lager AM, Zaremba A, Wyatt K, Caprariello AV, Factor DC, et al. Transcription factor-mediated reprogramming of fibroblasts to expandable, myelinogenic oligodendrocyte progenitor cells. *Nature biotechnology*. 2013;31(5):426.
27. 27. Guillemot F. Spatial and temporal specification of neural fates by transcription factor codes. *Development*. 2007;134(21):3771-80.
28. 28. Gao Z, Ure K, Ables JL, Lagace DC, Nave K-A, Goebbels S, et al. Neurod1 is essential for the survival and maturation of adult-born neurons. *Nature neuroscience*. 2009;12(9):1090.
29. 29. Deisseroth K, Singla S, Toda H, Monje M, Palmer TD, Malenka RC. Excitation-neurogenesis coupling in adult neural stem/progenitor cells. *Neuron*. 2004;42(4):535 – 52.
30. 30. Huang P, Kishida S, Cao D, Murakami-Tonami Y, Mu P, Nakaguro M, et al. The neuronal differentiation factor NeuroD1 downregulates the neuronal repellent factor Slit2 expression and promotes cell motility and tumor formation of neuroblastoma. *Cancer research*. 2011;71(8):2938-48.
31. 31. Sun Y, Nadal-Vicens M, Misono S, Lin MZ, Zubiaga A, Hua X, et al. Neurogenin promotes neurogenesis and inhibits glial differentiation by independent mechanisms. *Cell*. 2001;104(3):365 –

76.

32. 32. Ma Q, Sommer L, Cserjesi P, Anderson DJ. Mash1 and neurogenin1 expression patterns define complementary domains of neuroepithelium in the developing CNS and are correlated with regions expressing notch ligands. *Journal of Neuroscience*. 1997;17(10):3644-52.
33. 33. Sommer L, Ma Q, Anderson DJ. Neurogenins, a Novel Family of atonal-Related bHLH Transcription Factors, Are Putative Mammalian Neuronal Determination Genes That Reveal Progenitor Cell Heterogeneity in the Developing CNS and PNS. *Molecular and Cellular Neuroscience*. 1996;8(4):221 – 41.
34. 34. Masserdotti G, Gascón S, Götz M. Direct neuronal reprogramming: learning from and for development. *Development*. 2016;143(14):2494 – 510.
35. 35. Hardwick LJ, Philpott A. Multi-site phosphorylation regulates NeuroD4 activity during primary neurogenesis: a conserved mechanism amongst proneural proteins. *Neural development*. 2015;10(1):15.
36. 36. Braccioli L, Vervoort SJ, Puma G, Nijboer CH, Coffey PJ. SOX4 inhibits oligodendrocyte differentiation of embryonic neural stem cells in vitro by inducing Hes5 expression. *Stem cell research*. 2018;33:110-9.
37. 37. Wittstatt J, Reiprich S, Küspert M. Crazy Little Thing Called Sox—New Insights in Oligodendroglial Sox Protein Function. *International journal of molecular sciences*. 2019;20(11):2713.
38. 38. Stolt CC, Schlierf A, Lommes P, Hillgärtner S, Werner T, Kosian T, et al. SoxD proteins influence multiple stages of oligodendrocyte development and modulate SoxE protein function. *Developmental cell*. 2006;11(5):697–709.
39. 39. Martinez-Morales PL, Quiroga AC, Barbas JA, Morales AV. SOX5 controls cell cycle progression in neural progenitors by interfering with the WNT– β -catenin pathway. *EMBO reports*. 2010;11(6):466 – 72.
40. 40. Emery B, Lu QR. Transcriptional and epigenetic regulation of oligodendrocyte development and myelination in the central nervous system. *Cold Spring Harbor perspectives in biology*. 2015;7(9):a020461.

Figures

Figure 1

The 3'-UTR of Neurogenin, NeuroD1, NeuroD4, SOX4, SOX5 and SOX6 were cloned in the pSICHECK2 reporter vector and co-transfected with the pLEX-miR-19 vector in HEK293 cells for luciferase assays. For the construction of Mut-miR-19, two bases were mutated in the seed region of miR-19 and the mutated form of pre-miR-19 was cloned in pLEX. MiR-19 seed sequence is indicated in red color. The mutated forms of the bases were presented as a blue bold letter (A). MiR-19 expression significantly inhibited the luciferase activity of targets 3'-UTRs. Luciferase activity was detected at 48 h after transfection. The empty vector and Mut-miR-19 vectors were used as negative controls. MiR-19 overexpression inhibited luciferase activity in targets groups but did not affect the control. Data shown are mean values $\pm$ SD of at least three different experiments; (* $p < 0.05$) (B). Predicted model for miR-19 impact on differentiation of NPCs toward neural, astrocyte and OL cells. Neural progression inhibited by miR-19 overexpression

through targeting of key factors: Neurogenin, NeuroD1, NeuroD4, SOX4, SOX5 and SOX6. Furthermore, miR-19 not only changes the timing of NPCs differentiation via suppression of Neurogenin but also stimulated differentiation pathway toward OL lineage (C).

Figure 2

MiR-19 target genes prediction using the database for annotation, visualization and integrated discovery (DAVID) v6.8, based on the tissues in which they are expressed (A) and through interaction with their 3'-

UTR binding sites (B).

Figure 3

MiR-19 overexpression induced OL specific markers in NPCs were transduced with pLEX-Ctrl, pLEX-Scr or pLEX-miR-19, collected after 14 or 23 days and analysed with flow cytometry. Expression of OLIG2 and NKX2.2 increased in pLEX-miR-19 transduced group compared with the controls on day 14. MBP expression was observed only on day 23. There were no statistically significant changes between

obtained data of pLEX-Ctrl and pLEX-Scr groups at both time points (data not shown). The data shown in the figure is related to the control group that transduced with pLEX-Ctrl. Each experiment was performed in triplicates.

Figure 4

MiR-19 promoted OL differentiation in NPCs. MiR-19 expression evaluated by QRT-PCR in transduced NPCs with pLEX-Ctrl, pLEX-Scr, or pLEX-miR-19 relative to SNORD47 as an endogenous control (A). miR-19 overexpression reduced mRNA levels of non-OL specific genes and increased mRNA levels of OL specific genes after 7 days (B), 14 days (C) and 23 days post-transduction. β -Actin was used as an internal control in QRT-PCR. Columns represent mean of three different experiments; bars, $\pm$ SD; (* $p < 0.05$).

Supplementary Files

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