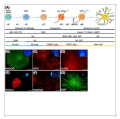

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Human embryonic stem cells differentiation into oligodendrocyte lineage cells

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ABSTRACT

Oligodendrocyte (OL) lineage cell generation from human embryonic stem cell line Royan H6 (RH6) started by dual inhibition of SMAD signaling. Therefore, within eight days, adherent NANOG$^+$RH6 cells (B) differentiated into SOX1$^+$ neural stem cells (C). Next, the embryonic ventral spinal cord environment (pMN domain) was mimicked by the application of two morphogens, RA and SAG and the OLIG2$^+$ neural progenitor cells were generated on day 12 (D) of differentiation. Further, oligodendrocyte progenitor cell commitment was achieved on day 20, when the pre-oligodendrocyte progenitor cell (pre-OPC) aggregates were positively stained by NKX2.2$^+$ (E). From day 20 to day 80, supportive reagents for pre-OPCs maturation into PDGFRA$^+$ OPCs (F), and their further expansion and maturation toward oligodendrocyte producing OPCs were added to the culture medium. Finally, on day 80 the growth-factors were withdrawn from the culture medium and OPCs were differentiated into MBP$^+$ OLs (G).

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MATERIALS TEXT

Media	Components	Provider	Final Conc.
NI medium	DMEM/F12	Life Technologies	
	Knock-Out Serum❊	Life Technologies	5%
	MEM Non-Essential Amino Acids (100X)	Life Technologies	1X
	GlutaMAX-I (100X)	Life Technologies	1X
	Penicillin-Streptomycin (100X)	Life Technologies	1X
	2-Mercaptoethanol (1000X)	Life Technologies	1X
	Insulin-Transferrin-Selenium (100X)	Life Technologies	1X
	N-2 Supplement (100X)	Life Technologies	1X
	SB431542	Stemgent	10 µM

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	LDN193189	Stemgent	250 nM
	All Trans-Retinoic Acid	Sigma	100 nM
N2 medium	DMEM/F12	Life Technologies	
	MEM Non-Essential Amino Acids (100X)	Life Technologies	1X
	GlutaMAX-I (100X)	Life Technologies	1X
	Penicillin-Streptomycin (100X)	Life Technologies	1X
	2-Mercaptoethanol (1000X)	Life Technologies	1X
	Insulin-Transferrin-Selenium (100X)	Life Technologies	1X
	N-2 Supplement (100X)	Life Technologies	1X
	All Trans-Retinoic Acid	Sigma	100 nM
	Agonist of Sonic Hedgehog (SAG)	Stemgent	1 µM
N2B27 medium	DMEM/F12	Life Technologies	
	MEM Non-Essential Amino Acids (100X)	Life Technologies	1X
	GlutaMAX-I (100X)	Life Technologies	1X
	Penicillin-Streptomycin (100X)	Life Technologies	1X
	2-Mercaptoethanol (1000X)	Life Technologies	1X
	Insulin-Transferrin-Selenium (100X)	Life Technologies	1X
	N-2 Supplement (100X)	Life Technologies	1X
	B-27 Supplement without Vitamin A (50X)	Life Technologies	1X
	All Trans-Retinoic Acid	Sigma	100 nM
	SAG	Stemgent	1 µM
PDGF medium	DMEM/F12	Life Technologies	
	MEM Non-Essential Amino Acids (100X)	Life Technologies	1X
	GlutaMAX-I (100X)	Life Technologies	1X
	Penicillin-Streptomycin (100X)	Life Technologies	1X
	2-Mercaptoethanol (1000X)	Life Technologies	1X
	N-2 Supplement (100X)	Life Technologies	1X
	B-27 Supplement without Vitamin A (50X)	Life Technologies	1X
	Insulin	Sigma	25 µg/ml
	PDGF-AA	Sigma	10 ng/ml
	HGF	Sigma	5 ng/ml
	IGF-1	Sigma	10 ng/ml
	NT3	Royan Biotech	10 ng/ml
	T3	Sigma	60 ng/ml
	Biotin	Sigma	100ng/ml
	cAMP	Sigma	1µM
Glial medium	DMEM/F12	Life Technologies	
	MEM Non-Essential Amino Acids (100X)	Life Technologies	1X
	GlutaMAX-I (100X)	Life Technologies	1X
	Penicillin-Streptomycin (100X)	Life Technologies	1X
	2-Mercaptoethanol (1000X)	Life Technologies	1X
	N-2 Supplement (100X)	Life Technologies	1X
	B-27 Supplement without Vitamin A (50X)	Life Technologies	1X
	Insulin	Sigma	25 µg/ml
	HEPES	Sigma	10 mM
	T3	Sigma	60 ng/ml
	Biotin	Sigma	100ng/ml
	cAMP	Sigma	1µM
	AA	Sigma	20 µg/ml

❊ From day four of differentiation, Knock-Out serum was gradually replaced by N-2 supplement (25%, 50%, 75%) as described previously.

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1 6×10^6 RH6 cells were plated on Engelbreth-Holm-Swarm mouse sarcoma ECM (sigma) coated 6cm cell culture plates on day -1.

2 On day 0, the FFC medium (containing 20% KSR and 100 ng/ml bFGF) was replaced by 4 ml NI medium. NI medium was refreshed every day.

3 On day 8, the NI medium was replaced with N2 medium and the medium was refreshed every day.

4 From day 4, 5% KSR was gradually withdrawn from the NI medium. Respectively, 3.75%, 2.5%, 1.25% and 0% KSR was applied in the NI medium on day 4, 5, 6 and 7.

5 On day 12, cells' attachment was loosened by PBS^- for 10 min. Then PBS^- was replaced with the N2B27 medium and cells were detached by braking their layer via the tip of the p1000 pipette. The cell clumps were pipetted gently with a p1000 pipette, for 5 times.

6 Detached cells were transferred into the 6 cm untreated plates to form aggregates and from day 14, two-thirds of the N2B27 medium was refreshed every day. The debris and dead cells were removed through the medium refreshment. Meanwhile, the aggregates that stuck to each other were broken apart by gentle pipetting (with the p1000 pipette).

7 On day 20, two-thirds of the N2B27 medium was replaced with PDGF medium. Two-thirds of the PDGF medium was refreshed every day until day 80.

8 On day 29, new 6 cm culture dishes were coated for overnight by 50 µg/ml poly-l-ornithine in dH2O, at 37 °C. Next, they were coated by 20 µg/ml laminin in PBS for 12 h at 37 °C.

9 On day 30, golden or brown aggregates (with a dark center) were plated on poly-l-ornithine/laminin coated dishes, with a p200 pipette (50 spheres per each 6 cm culture plate). The daily refreshment of the two-third of the PDGF medium was performed gently until day80.

10 FFirst on day 65 and then on day 75, cells and aggregates were re-plated on newly poly-l-ornithine/laminin coated dishes to achieve a more homogenous OPC population on day 80. PBS^- and the p200 pipette were used for detachment and replacement.

11 On day 80, the PDGF medium was replaced by the Glial medium. Two-thirds of the medium was refreshed every two to three days, until day 120.

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