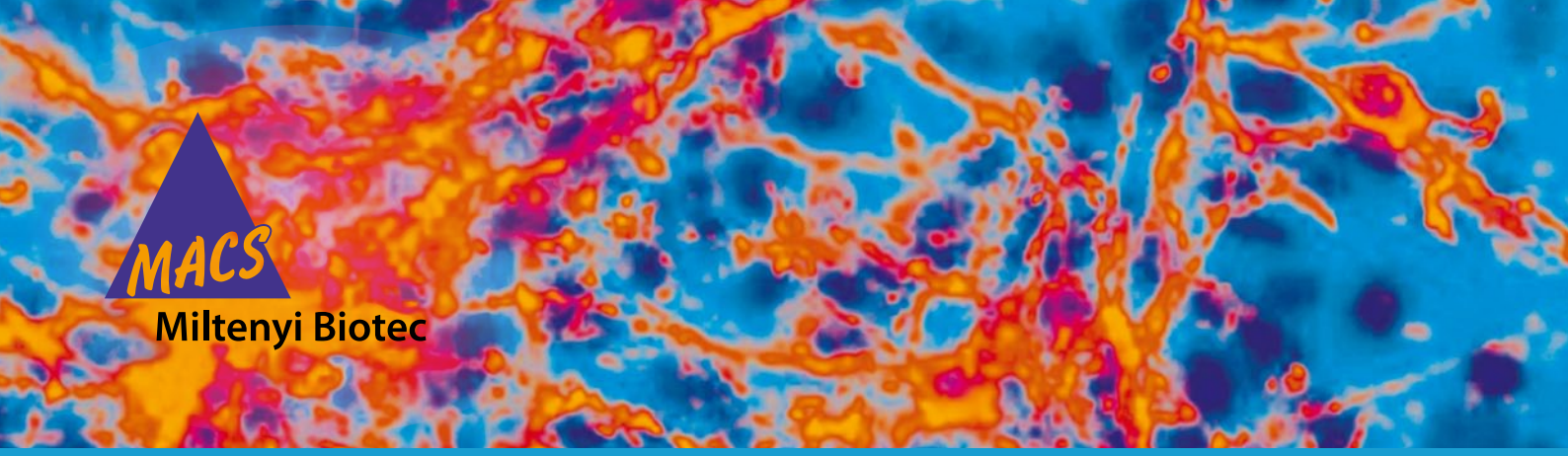

Enrichment of pluripotent stem cell derived neural crest stem cells and further differentiation to peripheral neurons

Background

Neural crest stem cells (NCSC) can be induced from pluripotent stem cells by synergistic action of two inhibitors of SMAD signaling, Noggin and SB431542[1,2] or alternatively, by a single small molecule dorsomorphin blocking the signaling of several (TGF-β) superfamily receptors.[3]
CD271$^+$ neural crest stem cells can be selected ten days post-induction. They show expression of typical neural crest markers (HNK1, AP2) and they can be differentiated to homogenous peripheral neurons.[4]
CD271, also known as LNGFR (low-affinity nerve growth factor receptor), NGFR (nerve growth factor receptor), or p75NTR (neurotrophin receptor), belongs to the tumor necrosis factor receptor superfamily. CD271 was initially described to be expressed on cells of the nervous system and was suggested to be involved in the development, survival and differentiation of neural cells.[5] CD271 can be found in the central and peripheral nervous system on autonomic and sensory neurons[6] as well as on glial cells, including oligodendrocytes[7], astrocytes[8], Schwann cells[9,10], and NCSCs[11].

Materials and methods

Note: For reagents, solutions, and materials required for the removal of feeder cells or the isolation of TRA-1-60$^+$ pluripotent stem cells and NCSCs, please refer to the data sheets of the Feeder Removal MicroBeads, mouse or Anti-Tra-1-60 MicroBead Kit, human and Neural Crest Stem Cell MicroBeads, human, respectively.

Reagents and solutions

PEB buffer: prepare a medium containing Dulbecco's Phosphate-Buffered Saline (DPBS), pH 7.2, 0.5% bovine serum albumin (BSA), and 2 mM EDTA.
NCSC differentiation medium: prepare a solution containing 80% DMEM/F12, 1x non-essential amino acids (100×), 1 mM L-glutamine, 0.1 mM ß-Mercaptoethanol, 20% KnockOut™ Serum Replacement, and 1% Penicillin/Streptomycin. Before use, add fresh 1 µM Stemolecule Dorsomorphin. Only for initial plating of the iPS cells, add fresh 2 µM Stemolecule Thiazovivin before use.
N-2 medium: prepare a medium containing 100% DMEM/F12, 1 mM L-glutamine, 1 × N-2 Supplement (100×), 0.1% MACS® NeuroBrew-21 w/o VitaminA, 20 µg/mL insulin , and 1% Penicillin/Streptomycin. Before use, add fresh 10 ng/mL Human FGF-2, premium grade and 10 ng/mL Human EGF, premium grade.
Peripheral neurons differentiation medium: prepare a medium containing 50% N-2 medium (without FGF-2 and EGF), 50% MACS Neuro Medium, and 1% MACS NeuroBrew-21 w/o VitaminA, Before use, add fresh 100 ng/mL cAMP, 10 ng/mL BDNF, 20 ng/mL NGF-β, and 100 µM ascorbic acid.

- Anti-Tra-1-60 MicroBead Kit, human (# 130-095-816) or Feeder Removal MicroBeads, mouse (# 130-095-531)
- Neural Crest Stem Cell MicroBeads, human (# 130-097-127)
- BD Matrigel™, hESC-qualified Matrix (BD, Cat.#354277)
- Poly-L-ornithin
- Laminin
- Fibronectin
- N-2 Supplement (100×) (PAA)
- KnockOut™ Serum Replacement (Life Technologies)
- Stemolecule Dorsomorphin (# 130-095-574)
- Stemolecule Thiazovivin (# 130-095-568)
- MACS NeuroBrew-21 w/o VitaminA (# 130-097-263)
- MACS Neuro Medium (# 130-093-570)
- Human FGF-2, premium grade (# 130-093-564)
- Human EGF, premium grade (# 130-097-749)
- Human BDNF (# 130-096-285)
- Human NGF-β (# 130-093-971)
- Ascorbic acid
- cAMP
- Insulin

Coating of cell culture plate with BD Matrigel™ for NCSC differentiation

Coat cell culture plate with BD Matrigel™ according to the manufacture's instructions. Briefly, thaw BD Matrigel™ over night at 4 °C. Use cold pipettes. Resuspend 150 µL BD Matrigel™ in 12 culture medium, e.g., DMEM or DMEM/F12 and pipette it onto the plate. Store plate over night at 4 °C. Remove the medium from the plate just before usage.

Coating of cell culture plate for cultivation and differentiation of NCSCs to peripheral neurons

1. Cover the plate with 100 µg/mL poly-L-ornithin in PBS. Incubate over night at 37 °C.
2. Remove the poly-L-ornithin/PBS from the plate
3. Wash two times with DPBS.
4. Cover the plate with 10 µg/mL laminin and 5 µg/mL fibronectin in PBS. Incubate over night at 4 °C.
5. Remove PBS shortly before usage.

Differentiation of induced pluripotent stem (iPS) cells to NCSCs

Note: Put BD Matrigel™ coated plate to room temperature one hour prior usage.

Harvest iPS cells cultured on feeder cells. Enrich human iPS cells using the Anti-Tra-1-60 MicroBead Kit, human or remove murine feeder cells using Feeder Removal MicroBeads, mouse. For detailed information on the protocols, please refer to the respective data sheets.

Note: To maintain the cells performance it is mandatory to perform all washing steps and the elution from the column with NCSC differentiation medium supplemented with 2 µM Stemolecule Thiazovivin. Optionally, collect cells by centrifugation after elution from the column. Resuspend cell pellet in NCSC differentiation medium supplemented with 2 µM Stemolecule Thiazovivin.

1. Determine the cell number and adjust the volume accordingly.
2. Aspirate the BD Matrigel™ solution from the plate.
3. Plate cells at density of 2.5×10^4–5×10^4 cells/cm^2 in NCSC differentiation medium containing 2 µM Stemolecule Thiazovivin.
4. Culture cells in NCSC differentiation medium for ten days. Renew medium every day.

Enrichment of CD271$^+$ NCSCs

Harvest of NCSCs differentiated from induced pluripotent cells (iPS). Enrich CD271$^+$ NCSCs using Neural Crest Stem Cell MicroBeads, human. For detailed information on the protocol, please refer to the respective data sheet.

Note: To increase the purity of CD271$^+$ cells, it is highly recommended to enrich the eluted fraction, containing CD271$^+$ neural crest stem cells, over a second MS Column.

Cultivation of CD271$^+$ NCSCs

1. Collect the isolated CD271$^+$ NCSCs by centrifugation. Resuspend cell pellet in N-2 medium.
2. Determine the cell number and adjust the volume accordingly.
3. Remove the PBS containing the laminin and fibronectin from the plate.
4. Plate cells at density of 5×10^4–1×10^5 cells/cm^2.
5. Cultivate NCSCs in N-2 medium. Renew medium every other day. Passage cells on day 4–7. For passaging, cells can be trypsinized according to standard procedures.

Differentiation towards peripheral neurons

1. When NCSCs are confluent, change medium to peripheral neuron differentiation medium.
2. Renew medium every 2–3 days.
3. The differentiation of NCSCs to peripheral neuron takes approximately 21 days.
4. After differentiation peripheral neurons can be further processed or analyzed.

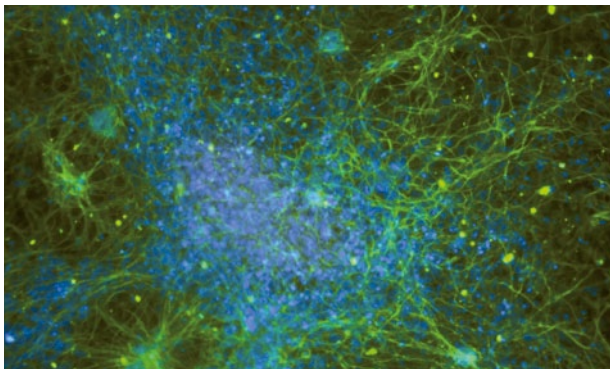

Figure 1: Peripheral neurons differentiated from enriched CD271$^+$ NCSCs. Cells were stained for peripherin. Nuclei were visualized using 4',6-diamidino-2-phenylindole (DAPI).

Publications

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