Video Article

Differentiation of Embryonic Stem Cells into Oligodendrocyte Precursors

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Abstract

Oligodendrocytes are the myelinating cells of the central nervous system. For regenerative cell therapy in demyelinating diseases, there is significant interest in deriving a pure population of lineage-committed oligodendrocyte precursor cells (OPCs) for transplantation. OPCs are characterized by the activity of the transcription factor Olig2 and surface expression of a proteoglycan NG2. Using the GFP-Olig2 (G-Olig2) mouse embryonic stem cell (mESC) reporter line, we optimized conditions for the differentiation of mESCs into GFP+Olig2+NG2+ OPCs. In our protocol, we first describe the generation of embryoid bodies (EBs) from mESCs. Second, we describe treatment of mESC-derived EBs with small molecules: (1) retinoic acid (RA) and (2) a sonic hedgehog (Shh) agonist purmorphamine (Pur) under defined culture conditions to direct EB differentiation into the oligodendroglial lineage. By this approach, OPCs can be obtained with high efficiency (>80%) in a time period of 30 days. Cells derived from mESCs in this protocol are phenotypically similar to OPCs derived from primary tissue culture. The mESC-derived OPCs do not show the spiking property described for a subpopulation of brain OPCs in situ. To study this electrophysiological property, we describe the generation of spiking mESC-derived OPCs by ectopically expressing $Na_V1.2$ subunit. The spiking and nonspiking cells obtained from this protocol will help advance functional studies on the two subpopulations of OPCs.

Video Link

The video component of this article can be found at http://www.jove.com/video/1960/

Protocol

Detailed procedures of generating oligodendrocyte precursor cells (OPCs) from GFP-Olig2 mouse embryonic stem cells (mESCs):

1. Mouse ES cell line GFP-Olig2 (G-Olig2), is purchased from the American Type Culture Collection (ATCC). The mESCs are routinely passaged every 3 days onto an irradiated mouse embryonic fibroblast (MEF) feeder layer. The MEF cells are plated into 0.1% gelatin-coated six-well tissue culture plate at least one day before passaging the ESCs. The mESC culture medium is Dulbecco's modified Eagle's medium (DMEM) (GIBCO) supplemented with 20% fetal bovine serum (GIBCO), 2 mM L-glutamine, 1 mM sodium pyruvate, 0.1 mM β-mercaptoethanol/, 1% nonessential amino acids (NEAA), and 1,000 U/ml leukemia inhibitory factor.
2. mES colonies are trypsinized (TrypLE, 5 min, 37°C) into single cells and suspended in knockout serum replacement (KSR) medium, then the cells are transferred to a Costar ultra-low attachment six-well plate (Corning) at a cell density of 50,000 cells/cm^2. In these conditions, mESCs form embryoid bodies (EBs). KSR medium consists of -minimal essential medium (-MEM) supplemented with 20% KSR, 1 mM sodium pyruvate, 1% NEAA, and 0.1 mM β-mercaptoethanol/.
3. From day 4 to day 7, retinoic acid (RA, 0.2 M) and purmorphamine (Pur, 1 M) are included as shown in Fig. 1A in KSR or N2 medium. The N2 medium is -MEM containing 1X N2 supplement, 1 mM sodium pyruvate, 1% NEAA, and 0.1 mM β-mercaptoethanol/. The medium is changed everyday.
4. At day 8, EBs are disaggregated using TrypLE (5min, 37°C) and plated on 0.01% polyornithine-coated dishes in OPC medium that consists of N2 medium and fibroblast growth factor-2 (FGF-2, 20 ng/ml). The medium is changed every two days.
5. The cells are trypsinized (TrypLE, 3 min, 37°C) and replated approximately every week when they become confluent. At day 30, over 80% of the cells show GFP/Olig2 expression and are NG2-positive, characteristic of OPCs.
6. To generate spiking OPCs, the BacMam Na^+ channel kit is used to introduce $Na_V1.2$ subunit into these mESC-derived OPCs. The BacMam reagent (1 ml, component A) is mixed with PBS to a final volume of 5 ml. Then, the mixed BacMam reagent is added into mESC-derived OPCs culture in a 60 mm culture dish (at 50-70% confluence) prior to rinsing with PBS. After 2 hr incubation, the mixed BacMam reagent is removed and replaced with OPC medium supplemented with 1X enhancer. The enhancer is the component B reconstituted in DMSO (component C). Next, after additional 2-hr incubation the medium with enhancer is removed and replaced with complete OPC medium. The cells are assayed 24 hrs later.

Representative Results:

Following the differentiation protocol shown in Fig. 1A, G-Olig2 mESCs were initially suspended in KSR medium and for 4 days to form embryoid bodies (EBs) (Fig. 1B). Then, we sequentially treated the EBs with RA and Pur. The EBs did not show any GFP fluorescence at Day 4 and expressed strong GFP fluorescence at D8 (Fig. 1B). At this time point, the EBs were trypsinized and plated in N2 medium supplemented with

growth factors FGF-2. After further culture for 22 days (D30), the percentage of GFP+ cell reached 80.3 ± 0.6 % according to our flow cytometry results. As shown in Fig. 1C, GFP+ cells overlapped with NG2 staining consistently (96.4 ± 1.3% of GFP positive cells were NG2 positive, and 94.8 ± 1.5% of NG2 positive cells were GFP positive). Thus, these cells express GFP/Olig2 and NG2, defining them as OPCs.

The mESC-derived OPCs (76 cells) failed to fire action potentials upon depolarization (Fig. 2A). After transducing with a baculovirus carrying the $Na_v1.2$ subunit, these mESC-derived OPCs (94.1%, 16 of 17 cells) could be classified as spiking (Fig. 2B).

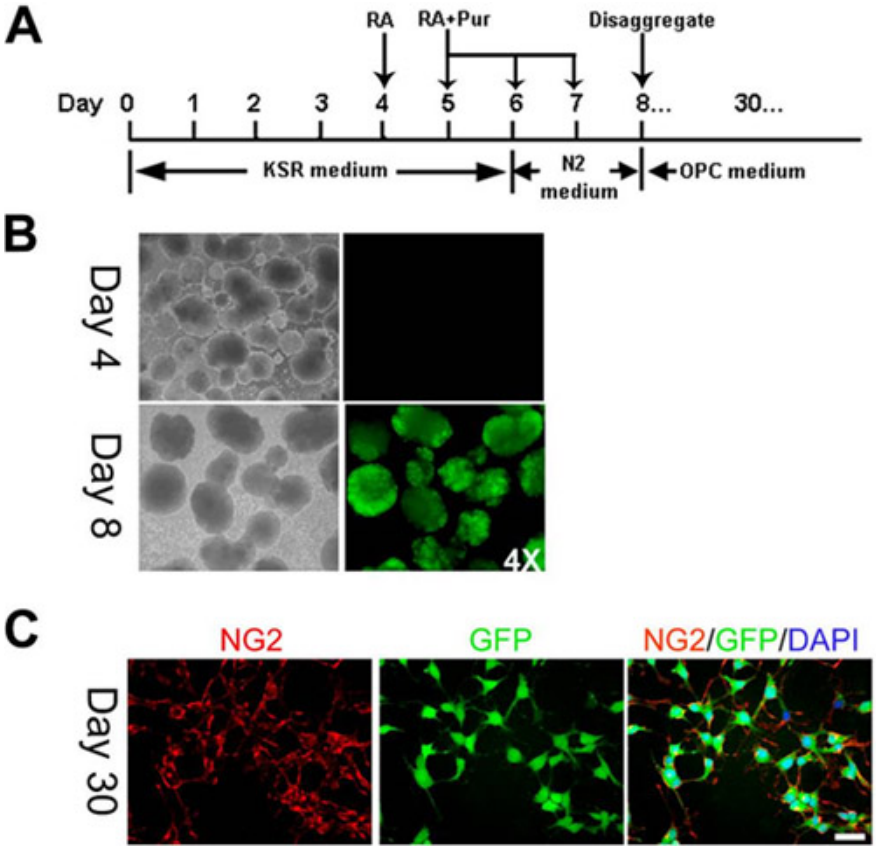

Figure 1. Differentiation of GOlig-mESC into OPCs. (A) Scheme showing the protocol of the embryoid body (EB)-based and small molecule-driven differentiation. At D8, the EBs were disaggregated and plated. The cells were passaged once per week when they became confluent. (B) D8, but not D4 EBs, showed GFP expression. (C) Immunostaining of NG2 (red) was consistent with GFP (green) expression at D30. DAPI (blue) was used to identify the nuclei. Scale bar: 20 mm.

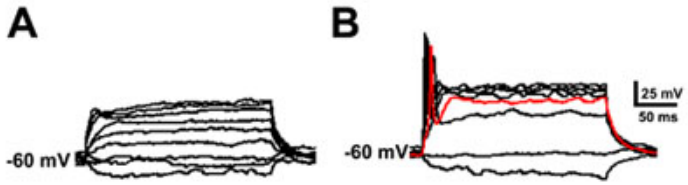

Figure 2. Generation of spiking OPCs from nonspiking mESC-derived OPCs by virus-mediated Na_v channel expression. (A) The membrane potential was held at -60 mV and mESC-derived OPCs failed to fire action potentials, even when the cell was depolarized to 0 mV. (B) An example of mESC-derived OPC firing action potential (highlighted in red) after the viral transduction.

Discussion

Embryonic stem cells (ESCs), isolated from a blastocyst embryo, can differentiate into all cell lineages of the organism [3, 4], providing an *in vitro* model system for studying early mammalian development, including oligodendrocyte specification. mESCs have been shown to differentiate into oligodendrocyte precursor cells (OPCs) with the treatment of sonic hedgehog (Shh) [5]. Moreover, the Shh-induced OPC differentiation from mESCs retains the correct timing observed in embryonic development [6]. Hence, the nature of *in vitro* OPC differentiation from mESCs is considered to be consistent with what has been learned from *in vivo* development. Here, using the small molecules RA and the Shh agonist Pur, we successfully differentiated the G-Olig2 mESCs into GFP+Olig2+NG2+ OPCs with high efficiency.

OPCs, characterized by the expression of the proteoglycan NG2 [7] and the helix-loop-helix transcription factor Olig2 [8], generate oligodendrocytes in the developing and mature CNS, where they comprise a significant percentage (~ 5%) of the total cells and are the main proliferating cell type [9]. For nearly two decades researchers have demonstrated Na_V channels are expressed in a subpopulation of OPCs and can be activated upon

depolarization [10, 11]. Moreover, a recent striking observation[9] was that in *in situ* rat CNS white matter, OPCs (~ 50%) generated action potentials when depolarized depending upon the expression of voltage-gated sodium (Na_V) channels, and thus could be subdivided into spiking and nonspiking subpopulations. However, the functions of these spiking properties are still largely unknown. We found that, electrophysiologically, the mESC-derived OPCs differentiated with the Shh-dependent protocol, were not the same as the *in situ* brain OPCs. After introducing subunit $Na_V1.2$, these silent mESC-derived OPCs were capable of spiking.

Thus, the spiking/nonspiking mESC-derived OPCs and differentiation protocol described here may facilitate (1) studying the functional differences between spiking and nonspiking OPCs, (2) screening new factors that could promote sodium channel expression in the mESC-derived OPCs, (3) developing and optimizing the differentiation protocol of OPCs from human ESC or induced pluripotent stem cells (iPSCs).

Disclosures

No conflicts of interest declared.

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