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Spontaneous Generation of Neurospheres from Mouse Embryonic Stem Cells

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1. Introduction

Neural stem cells (NSCs) are self-renewing pluripotent cells that can produce different parts of the nervous system. NSCs were initially identified in the subventricular zone of the mouse brain (Temple, 1989) and subsequently in various regions of adult brains from human and mouse (Taupin & Gage, 2002). NSCs can be derived in vitro from embryonic stem (ES) cells and induced pluripotent stem (iPS) cells by employing specific culture conditions. NSCs and their progeny can be expanded for several passages as tridimensional floating aggregates named neurospheres or as monolayer cultures. This in turn allows derivation of neurons of different parts of the nervous system (Gaspard & Vanderhaeghen, 2011). NSCs derived from ES/iPS cells of various genetic backgrounds represent invaluable tools for the investigation of neurogenesis, development of neurologic diseases models, and screening of new drugs to treat neurological diseases.

Derived from ES cells, NSCs and their progeny, neural progenitors, are routinely studied in vitro by a method called neurosphere culture system (Reynolds & Weiss, 1992). Neurospheres can measure 100-300 mm. The zonal distribution of different cell types that compose human and murine neurospheres resembles an outside-in brain structure with nestin-positive progenitor cells in the periphery and GFAP+ and β-tubulin III-positive cells in the centre (Moors et al., 2009; Campos et al., 2004).

Currently, there are two methods that allow derivation of neurospheres from mouse ES cells. Both of these methods utilize fibroblast growth factor 2 (FGF-2), epidermal growth factor (EGF), retinoic acid, and other supplements such as B27 and N2. FGF-2 is a critical component in neural differentiation protocols as it promotes proliferation of neuroprogenitor cells (Yoshimura et al., 2001). For selecting and expanding neural progenitor cells, culture media are supplemented with B-27 (containing retinoic acid) and N2 (which has a subset of component of B-27 that include insulin and transferrin).
In one method of derivation, ES cells are cultured in a specific medium supplemented with EGF, FGF-2 and N2. After 4-5 days, 50-80% of the cells undergo neural lineage specification. On day 7, these cells are dissociated and plated in suspension in uncoated plates in a differentiation medium supplemented with N2, B27, mouse EGF and human FGF-2. After a few days of culturing, neurospheres can be obtained (Conti et al., 2005).

Alternatively, in a second method, mouse ES cells are co-cultured for approximately one week with the PA6 stromal cell line to achieve efficient neuronal differentiation because of the stromal cell-derived inducing activity (SDIA) (Kawasaki et al., 2000). After 8 days of SDIA treatment, mouse ES cells are separated from PA6 stromal cells and cultured in suspension in serum-free medium supplemented with FGF-2. Under these conditions neurospheres appear within 4 days (Morizane et al., 2006).

Exemplifying stages of the neurosphere generation process are shown in figure 1. The ability of mouse ES cells to spontaneously generate neurospheres and mature neurons in short time and in few steps, represents the strength for a powerful and reliable in vitro model to apply research strategies oriented to study the physiopathology of the biochemical and epigenetic mechanisms leading to the neurogenesis.

We recently observed that mouse ES cells when grown in simple culture conditions spontaneously form neurospheres. In this chapter, we describe a simple method for derivation of neurospheres from mouse ES cells without using FGF-2 and EGF and without co-culturing ES cells with stromal cells.

Fig. 1. Generation of neurospheres from mES cells. a) Microphotograph of a mES colony; b) typical clusters of cells generated after culturing R1 mES for three passages in a DMEM/FBS medium; c) bright field of a neurosphere; d) progenitors migrating from neurospheres. [Images taken by authors.] Scale bar 1s 100 mm.
2. Reagents and equipment

The reagents and equipment needed for the methods described in this chapter can be found listed in the tables below.

| Equipment                                                                                                | Company          | Catalog # |
|----------------------------------------------------------------------------------------------------------|------------------|-----------|
| 0.22-μm 500 ml bottle top filter                                                                         | Corning          | 430513    |
| 0.22-μm 250 ml filter system                                                                             | Corning          | 430767    |
| 10 cm cell culture dish                                                                                   | Corning          | 439167    |
| 24-well plates (Transwell-Clear permeable support 0.4 μm)                                                 | Fischer          | 07-200-154|
| 6-well ultra low attachment plate                                                                       | Costar           | 3471      |
| 15-ml polypropylene conical tubes                                                                         | BD Falcon        | 352096    |
| 50-ml polypropylene conical tubes                                                                         | BD Falcon        | 532070    |
| Transfer pipettes                                                                                         | Samco            | 262-1S    |
| 0.22-μm bottle top filters                                                                               | TPP              | 99505     |
| 0.45-μm bottle top filters                                                                               | Nalgene          | 165-0045  |
| 12 mm circular cover slips                                                                              | Fisher           | 01-472A   |
| Hemocytometer                                                                                             | Hycor Kova       | 87144     |
| 1000-ml media bottles                                                                                    |                  |           |
| Kimwipes                                                                                                 | Kimberly-Clarks  | 34155     |
| Humidified tissue culture incubator (37°C, 5% CO₂)                                                        |                  |           |
| Centrifuge                                                                                               |                  |           |
| Water bath (37°C)                                                                                        |                  |           |
| Scanning confocal microscope                                                                             | Olympus          | FluoView 100 |
| DM IRM inverted microscope (fitted with Leica HS N Plan BD 50X oil immersion objective)                  | Leica            | -         |
| 75W xenon lamp-based monochromator                                                                        | Ushio, Japan     | -         |
| CCD camera                                                                                                | Orca (Hamamatsu, Shizouka, Japan)                        | -         |
| 535 ± 40nm bandpass filter                                                                               | Omega Optical    | -         |
| 500 nm longpass dichromatic mirror                                                                        |                  |           |
| Simple PCI                                                                                                | Compix, Inc (Cranberry, PA)                             | -         |

Table 1. Equipment needed for the methods described in this chapter.
| Reagent                                                                 | Company                      | Catalog #                  |
|------------------------------------------------------------------------|------------------------------|----------------------------|
| R1 ES cells                                                            | Mutant Mouse Regional        | 011979-MU                  |
| Non-essential amino acid solution (100X)                               | Millipore                    | TMS-001                    |
| Nucleosides (100X)                                                     | Millipore                    | ES-008-E                   |
| L-glutamine (100X)                                                     | Millipore                    | TMS-002-C                  |
| Penicillin/Streptomycin (100X)                                         | Millipore                    | TMS-001                    |
| 2-Mercaptoethanol (100X)                                               | Millipore                    | ES-007-E                   |
| Sodium pyruvate (100mM)                                                | Celigro                       | 25-00-CI                   |
| Trypsin-EDTA (100X)                                                    | Gibco                        | 15400                      |
| Dulbecco’s phosphate buffered saline (10X) (PBS)                       | Sigma                        | P-3813                     |
| Gelatin from porcine skin (Type A)                                     | Sigma                        | G1890                      |
| Dulbecco’s modified Eagle’s medium (DMEM)                              | Millipore                    | SLM-220-B                  |
| Fetal bovine serum (ES cell grade) (FBS)                               | Gibco                        | 16141-079                  |
| Knockout Serum Replacement (KSR)                                       | Invitrogen                   | 10828028                   |
| Neurobasal medium                                                      | Invitrogen                   | 21103-049                  |
| Phosphate buffered saline (PBS)                                        | Gibco                        | 14040141                   |
| Poly-D-lysine (PDL) hydrobromide                                       | Sigma                        | P6407                      |
| Leukemia inhibitory factor (LIF) (10^7)                                | Millipore                    | ESG1107                    |
| B27                                                                    | Invitrogen                   | 17504-044                  |
| N2 supplement                                                          | Invitrogen                   | 17502-048                  |
| FGF-basic Recombinant Mouse                                           | Invitrogen                   | PMG0035                    |
| Laminin                                                               | Sigma                        | L2020                      |
| BisBenzimide H 33342 trihydrochloride                                  | Sigma                        | B-2261                     |
| Mounting solution                                                      | Gelvatol                     | -                          |
| Paraformaldehyde (16% in premixed PBS buffer at pH7 [below])           | Electron Microscopy Sciences | 15710-6                    |
| PBS buffer at pH7 (1X)                                                 | Roche                        | 11666789001                |
| 10% Normal Donkey Serum (NDS)                                          | Jackson ImmunoResearch       | 711-165-152                |
| 0.01% TritonX-100                                                      | Fluka                        | BP151-100                  |
| Tubulin III                                                            | R&D Systems                  | MAB1195                    |
| Rabbit Anti-NMDAR1                                                    | Abcam                        | Ab68144                    |
| Bovine albumin                                                         | Sigma                        | A-6003                      |
| Fura-2, AM cell-permeant                                              | Invitrogen                   | F-1221                     |
| Cy3 AffiniPure Donkey Anti-Rabbit                                      | Jackson ImmunoResearch       | 711-165-152                |
| AffiniPure Donkey Anti-Mouse                                          | Jackson ImmunoResearch       | 715-175-151                |

Table 2. Reagents needed for the methods described in this chapter.
3. Reagent and equipment setup

The setup for the reagents and equipment listed is as follows.

| Reagent                                | Setup                                                                                                                                                                                                 |
|----------------------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| ES medium                              | For 500 ml of ES medium, add the following components: 400 ml DMEM, 75 ml FBS, 5 ml Pen/Strep, 5 ml 2ME (100 mM), 5 ml L-glutamine, 5 ml NEAA, 5 ml nucleosides, 5 ml 2ME, and 50 μl LIF.                        |
| EB medium                              | For 500 ml of EB medium, add the following components: 450 ml DMEM, 25 ml KSR, 5 ml sodium pyruvate, 5 ml Pen/Strep, 5 ml 2ME (100 mM), 5 ml L-glutamine, and 5 ml NEAA.                                             |
| DMEM/FBS medium                        | For 500 ml of feeder cells medium, add the following components: 440 ml DMEM, 50 ml FBS, 5 ml NEAA, and 5 ml Pen/Strep.                                                                                      |
| N2 medium                              | For 100 ml of N2 medium, add 96 ml DMEM/F12, 1 ml N-2, 1 ml Pen/Strep, 1 ml L-glutamine, 1 ml NEAA, 4 μl FGF-2, and 100 μl laminin.                                                                              |
| B27/Neurobasal medium                  | For 500 ml of B27/Neurobasal medium, add the following components: 480 ml Neurobasal medium, 10 ml B27, 5 ml L-glutamine 100 X, and 5 ml Pen/Strep.                                                      |
| Gelatin 0.1%                           | Dissolve 100 mg of gelatin in 90 ml warm (~60 °C) distilled water. Sterilize by autoclaving and store at room temperature.                                                                                  |
| 25x FGF stock solution (100 μg ml⁻¹)   | Dissolve 25 mg recombinant mouse FGF in 250 ml of sterile PBS. Store at ~20 °C.                                                                                                                      |
| 5x Poly-D-Lysine                       | Dissolve 50 mg poly-D-lysine in 333.3 ml distilled water and rinse the package with the water. Filter the solution with a 0.22-µm bottle top filter. Aliquot 5 ml into 15-ml conical tubes and store at ~20 °C. |
| HEPES-buffered salt solution (HBSS)    | Prepare stocks of the composition described in table 4 below. To obtain the HBSS basic, combine the solutions in the following order: 10.0 ml Solution #1, 1.0 ml Solution #2, 1.0 ml Solution #3, 1.0 ml Solution #5, 86.0 ml distilled H₂O, 1.0 ml Solution #4. Prior to use, prepare the following solution: 9.8 ml HBSS basic, 0.1 ml Stock #5, 0.1 ml Stock #6. |

The final HBSS composition is 137 mM NaCl, 5.4 mM KCl, 0.6 mM Na₂HPO₄, 0.6 mM KH₂PO₄, 20 mM KH₂PO₄, 1.3 mM CaCl₂, 1.0 mM MgSO₄, 10 mM NaHCO₃, 5.5 mM glucose.

Table 3. Setup of reagents needed for the methods described.
Table 4. Preparation of the stock solutions needed to prepare HBSS. The combination of these stock solutions in described in table 3.

| Stock | Preparation |
|-------|-------------|
| 1     | In 90ml of distilled H₂O, dissolve 8.0 g NaCl. 0.4 g KCl, and fill to 100 ml with distilled H₂O. |
| 2     | In 90ml of distilled H₂O, dissolve 0.41 g Na₂HPO₄ (anhydrous), 0.80 g KH₂PO₄, and fill to 100 ml with distilled H₂O. |
| 3     | Add 0.72 g CaCl₂ to 50ml of distilled H₂O. |
| 4     | Add 1.23 g MgSO₄7H₂O to 50ml of distilled H₂O. |
| 5     | Add 4.76 g Hepes to 10ml of distilled H₂O. |
| 6     | Add 0.9 g Glucose to 10ml of distilled H₂O. |
| 7     | Add 0.84 g NaHCO₃ to 10ml of distilled H₂O. |

Table 5. Setup for the equipment needed for the methods described.

| Equipment                  | Setup                                                                 |
|----------------------------|-----------------------------------------------------------------------|
| Gelatin-coated plate       | Add 5 ml of 0.1% (wt/vol) gelatin solution into a plate so that it covers the entire bottom of the plate. Incubate the plate for 20 min at room temperature. |
| Poly-D-lysine (PDL) cover slips | Autoclave cover slips. Place sterilized cover slips into the wells of a 24-well plate. Dilute the 5 x poly-D-lysine to 1 x with sterile distilled water plate except for the outermost wells. Add 0.5–1 ml of the 1 x poly-D-lysine solution to cover the surface of the cover slips. After an overnight incubation, remove the PDL and wash the plates with sterile deionized water and dry in a cell culture hood. |

4. Methods

The generation of neurospheres and subsequent neuronal differentiation from ES cells is achieved through the stages described in the protocols below.

4.1 Maintenance of ES cells

Place R1 ES cells (Nagy et al., 1993) on 10 cm gelatin-coated dishes with 12 ml of ES medium in a humidified chamber in a 5% CO₂/air mixture at 37°C. Change the medium every day. When the cells reach 80-90% confluence split them to two dishes as follows: wash the cells with 1x PBS twice, then add 2 ml of 1x trypsin-EDTA. Rock the dish to ensure that the solution covers all the cells. Incubate the dish for 5 min at 37°C. Pipette up and down to break up clumps and obtain a good dissociation of cells. Pipette 8 ml ES medium to inhibit further trypsin activity and transfer to 15-ml conical tubes. Spin at 1000 rpm for 3 min. Aspirate the medium from cells and resuspend the pellet in 6 ml of fresh ES medium. Transfer 3 ml of cell suspension into a new 10 cm gelatin-coated dish containing 9 ml of fresh ES medium.

4.2 Generation of embryoid bodies

Aspirate the medium, add 2 ml of 1x trypsin-EDTA and incubate for 5 min at 37 °C. Dissociate the cells by thoroughly pipetting several times with a P1000 Pipetman. Monitor
the trypsinization under an inverted microscope. If the separation of the cells is not complete, continue the treatment with trypsin for additional 2-3 min. Transfer the cell suspension into a 15-ml sterile conical tube. Pellet the cells centrifugation for 5 min at 1200 rpm; then resuspend the cell pellet in 10 ml EB medium. Count the cells with a cell counter and plate $5 \times 10^5$ ES cells per well in 6-well ultra low attachment plates in EB medium. Embryoid bodies should appear by overnight incubation. Culture plates for 7 days. Change the medium every other day as follows: Collect the EBs in suspension from the plate and transfer into a 15 ml conical tube. Leave the conical tube for 15 minutes to allow the EBs to settle to the bottom of the tube. Aspirate the medium and add 12 ml of fresh EB medium. Transfer the EBs suspension into a new 6-well ultra low attachment plates.

4.3 Neurosphere generation and neuronal differentiation

Collect EBs and remove the medium as described in the previous step. Count the number of EBs and prepare an EBs suspension in Feeder medium at a density of 40-50 EBs/ml. Transfer 40-50 intact EBs to non-coated 10 cm cell culture dish containing 12 ml DMEM/FBS medium. Culture until cells reach confluence, changing medium every 2-3 days. Aspirate the medium, add 2 ml of trypsin-EDTA solution and incubate for 5 min at 37°C. Dissociate cells thoroughly by pipetting with a P1000 Pipetman. Pipette 8 ml Feeder medium and transfer the cell suspension to a 15-ml conical tube. Centrifuge for 5 min at 1200 rpm. Aspirate the medium and resuspend the cells in 12 ml DMEM/FBS. Culture the cells until confluence. Trypsinize as described in section 4.2 and culture cells for other two passages. Transfer the neurospheres floating in the culture medium into 50-ml conical tubes and allow them to settle for 10 minutes. Collect the supernatant containing neurospheres. Trypsinize cells attached to the bottom of the plate as described in section 6.2 and culture to generate new neurospheres.

4.4 Neuronal differentiation

Resuspend the neurospheres in the appropriate volume of N2 medium and culture for differentiation in a 24-well plate (15-20 neurospheres/well) containing poly-D-lysine-coated cover slips. Replace a half volume medium every other day. At day 7 exchange N2 medium to B27/Neurobasal medium and let the cells differentiate for 10 more days with a medium change every other day.

5. Characterization and functional analysis of neurons

Selective immunocytochemistry can be performed according to standard procedures as described in the following sections.

5.1 Cell cultures
Plate the neurospheres on PDL-treated cover slips, and culture according to experimental protocols with the same conditions described above.
5.2 Immunocytochemistry procedure
Fix mouse neurospheres-derived neurons for 20 minutes at room temperature in a 4% paraformaldehyde solution (prepared from stock 16% Electron Microscopy Sciences, 15710-6 in 1X Premixed PBS Buffer at pH 7: Roche, 11666789001) after three 10-minutes washes in PBS at room temperature, perform a blocking step for 1 hour in 10% of Normal Donkey Serum (NDS, Jackson ImmunoResearch, 017-000-121) supplemented with 0.01% TritonX-100 (Fluka, BP151-100). Incubate the samples overnight with primary antibodies diluted in 10% of NDS in order to detect the expression of Tubulin III (R&D Systems, MAB1195) and of the ubiquitous NMDA receptor subunit NR1. (Rabbit Anti-NMDAR1, Abcam ab68144; 1/250).
After three 10-minutes washes in PBS at room temperature, use fluorescently labeled secondary antibodies for detection (Cy3 AffiniPure Donkey Anti-Rabbit [Jackson ImmunoResearch, 711-165-152] or Cy5 AffiniPure Donkey Anti- Mouse [Jackson ImmunoResearch, 715-175-151]).

5.3 Intracellular Ca^{2+} measurement
For intracellular Ca^{2+} concentration ([Ca^{2+}]) measurements, incubate cells for 45 min at 37°C with 5 µM fura-2-AM (cell permeant; Invitrogen, F-1221) and 5 µg/ml bovine serum albumin (Sigma A-6003) in Hepes-buffered salt solution (standard HBSS). Then, wash cells 3 times for 5 min each with standard HBSS and place the cover slips onto a perfusion chamber on a DM IRM inverted microscope (Leica) fitted with a Leica HC N PLAN BD 50X oil immersion objective. [Ca^{2+}], can be monitored in single cells using excitation light provided by a 75W xenon lamp-based monochromator (Ushio, Japan). Emitted light can be detected using a CCD camera (Orca; Hamamatsu, Shizouka, Japan). Alternatively, illuminate cells with 340 and 380 nm light for fura-2. Emitted fluorescence can be passed through a 500 nm longpass dichromatic mirror and a 535 ± 40 nm bandpass filter (Omega Optical). Analyze acquired data using software such as Simple PCI (Compix, Inc., Cranberry, PA) as the 340/380 ratio. Measure fluorescence in 15–25 individual neurons for each cover slip. Subtract background fluorescence, determined from three or four cell-free regions of the cover slips, from all signals prior to calculating the ratios. Choose the excitation light exposure time and a neutral density filter to avoid saturation of the fluorescence signal.

6. Conclusion
Neural induction from pluripotent stem cells often yield heterogeneous cell populations that can hamper quantitative and comparative analyses. There is a need for improved differentiation and enrichment procedures that generate highly pure populations of neural stem cells (NSC), glia and neurons. The method described here allows an efficient generation of neurospheres from mES cells. On day-in vitro 16 after neural induction about 50% of the cells derived from neurospheres show a neural morphology, a significant
expression of neuronal markers such as TuJ1 and the ubiquitous NMDA receptor subunit NR1 (figure 2), and a functional NMDAR-dependent calcium influx mediated by exogenous glutamate administration (figure 3).

**Fig. 2.** Neurosphere differentiation. a) Immunostaining of a neurospheres with antibody specific to NMDA receptor (NR1 sub-unit, green). b-c) Staining of neurosphere-derived neurons with (b) NMDA receptor (NR1 sub-unit, red) and (c) β-tubulin III (green). d) staining of astrocytes differentiated from neurospheres with GFAP (red). Nuclei were counterstained with DAPI (blue). [Images taken by authors.]

**Fig. 3.** Analysis of calcium influx in neurons differentiated from neurospheres indicated by glutamate. a) Administration of 10μM glutamate (glu) in neurons causes an increase in $[\text{Ca}^{2+}]_i$ (340/380 = 0.32 ± 0.05a). b) Glu-induced increase in $[\text{Ca}^{2+}]_i$ in R1 cells is blocked by treatment with MK801, an NMDAR-specific antagonist, suggesting that NMDARs are the predominant glutamate-sensitive receptors in neurospheres-derived neurons. [Data generated by authors.]
Furthermore, the rest of the cellular population show typical astroglial morphology. This chapter illustrates the utility of mouse ES cells as a simple system able to develop populations of viable NSCs and neurons. The method described here will enable downstream studies that require consistent and defined cell populations to study and characterize the physiopathology of the neuronal differentiation.

This procedure allows generation of a cell line derived from mouse ES cells which continually produces neurospheres in the absence of FGF-2 and EGF and coculturing. By generating neurospheres via this method, one can use them for a variety of purposes for further experiments with neurons.

9. References

Campos LS., Leone DP., Relvas JB., Brakebusch C., Fassler R., Suter U., et al. (2004). Beta1 integrins activate a MAPK signalling pathway in neural stem cells that contributes to their maintenance. *Development*, Vol. 131, No. 14 (July 2004), pp. 3433–3444. ISSN 1011-6370.

Conti L., Pollard SM., Gorba T., Reitano E., Toselli M., Biella G., Sun Y., Sanzone S., Ying QL., Cattaneo E. & Smith A. (2005). Niche-independent symmetrical self-renewal of a mammalian tissue stem cell. *PLoS Biol*, Vol. 3, No. 9 (Sep 2005)pp. e283. ISSN 1545-7885.

Gaspar N. & Vanderhaeghen P. (2011) From stem cells to neural networks: recent advances and perspectives for neurodevelopmental disorders. *Dev Med Child Neurol*, Vol. 53, No. 1 (Jan 2011), pp. 13-7. ISSN 0012-1622.

Kawasaki H., Mizuseki K., Nishikawa S., Kaneko S., Kuwana Y., Nakanishi S., Nishikawa SI. & Sasai Y. (2000). Induction of midbrain dopaminergic neurons from ES cells by stromal cell-derived inducing activity. *Neuron*, Vol. 28, No. 1 (Oct 2000), pp. 31-40. ISSN 0986-6273.

Moors M., Rockel TD., Abel J., Cline JE., Gassmann K., Schreiber T., Schuwald J., Weinmann N. & Fritsche E. (2009). Human neurospheres as three-dimensional cellular systems for developmental neurotoxicity testing. *Environmental Health Perspectives*, Vol. 117, No. 7 (July 2009), pp. 1131-8. ISSN 0091-6765.

Morizane A., Takahashi J., Shinoyama M., Ideguchi M., Takagi Y., Fukuda H., Koyanagi M., Sasai Y. & Hashimoto N. (2006). Generation of graftable dopaminergic neuron progenitors from mouse ES cells by a combination of coculture and neurosphere methods. *J Neurosci Res*, Vol. 83, No. 6 (May 2006), pp. 1015-27. ISSN 0306-4522.

Nagy A., Rossant J., Nagy R., Abramow-Newerly W. & Roder JC. (1993). Derivation of completely cell culture-derived mice from early-passage embryonic stem cells. *Proc Natl Acad Sci USA*, Vol. 90, No. 18, (Sep 1993), pp. 8424-8428. ISSN 0027-8424.

Reynolds BA & Weiss S. (1992). Generation of neurons and astrocytes from isolated cells of the adult mammalian central nervous system. *Science*, Vol. 255, No. 5052 (Mar 1992), pp. 1707-10. ISSN 0193-4511.

Taupin P. & Gage FH. (2002). Adult neurogenesis and neural stem cells of the central nervous system in mammals. *J Neurosci Res*, Vol. 89, No. 6 (Sep 2002), pp. 745-9. ISSN 0360-4012.

Temple S. (1989). Division and differentiation of isolated CNS blast cells in microculture. *Nature*, Vol. 340, No. 6233 (Aug 1989), pp. 471-3. ISSN 0028-0836.

Yoshimura S., Takagi Y., Harada J., Teramoto T., Thomas SS., Waebser C., Bakowska JC., Breakefield XO. & Moskowitz MA. (2001). FGF-2 regulation of neurogenesis in adult hippocampus after brain injury. *Proc Natl Acad Sci U S A*, Vol. 98, No. 10, (May 2001), pp. 5874-9. ISSN 0027-8424.
Based on our current understanding of cell biology and strong supporting evidence from previous experiences, different types of human stem cell populations are capable of undergoing differentiation or trans-differentiation into functionally and biologically active cells for use in therapeutic purposes. So far, progress regarding the use of both in vitro and in vivo regenerative medicine models already offers hope for the application of different types of stem cells as a powerful new therapeutic option to treat different diseases that were previously considered to be untreatable. Remarkable achievements in cell biology resulting in the isolation and characterization of various stem cells and progenitor cells has increased the expectation for the development of a new approach to the treatment of genetic and developmental human diseases. Due to the fact that currently stem cells and umbilical cord banks are so strictly defined and available, it seems that this mission is investigational more practical than in the past. On the other hand, studies performed on stem cells, targeting their conversion into functionally mature tissue, are not necessarily seeking to result in the clinical application of the differentiated cells; In fact, still one of the important goals of these studies is to get acquainted with the natural process of development of mature cells from their immature progenitors during the embryonic period onwards, which can produce valuable results as knowledge of the developmental processes during embryogenesis. For example, the cellular and molecular mechanisms leading to mature and adult cells developmental abnormalities are relatively unknown. This lack of understanding stems from the lack of a good model system to study cell development and differentiation. Hence, the knowledge reached through these studies can prove to be a breakthrough in preventing developmental disorders. Meanwhile, many researchers conduct these studies to understand the molecular and cellular basis of cancer development. The fact that cancer is one of the leading causes of death throughout the world, highlights the importance of these researches in the fields of biology and medicine.

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