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MINIREVIEWS

Pluripotent stem cell-derived neural stem cells: From basic research to applications

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Abstract

Basic research on pluripotent stem cells is designed to enhance understanding of embryogenesis, whereas applied research is designed to develop novel therapies and prevent diseases. Attainment of these goals has been enhanced by the establishment of embryonic stem cell lines, the technological development of genomic reprogramming to generate induced-pluripotent stem cells, and improvements in *in vitro* techniques to manipulate stem cells. This review summarizes the techniques required to generate neural cells from pluripotent stem cells. In particular, this review describes current research applications of a simple neural differentiation method, the neural stem sphere method, which we developed.

Key words: Pluripotent stem cells; Embryonic stem cells; Neural stem cells; Neural Stem Sphere method; Cell-based therapies

Core tip: *In vitro* techniques for manipulating stem cells can enhance the development of stem cell-based therapies and effective prevention against human diseases. This review summarizes the techniques required to generate neural cells from pluripotent stem cells, as well as focusing on current research applications of a simple neuronal differentiation method, the neural stem sphere method.

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INTRODUCTION

All somatic cells forming an individual are derived from one fertilized egg, a totipotent stem cell, which differentiates into preimplantation blastocysts that possess a pluripotent inner cell mass (ICM). Pluripotency is defined as the potential to differentiate into any somatic cell *via* three embryonic germinal layers: the endoderm, the mesoderm and the ectoderm. Mechanisms of pluripotency have been studied in embryonal carcinoma (EC) cells as *in vitro* models[1,2]. Although EC cells have some properties similar to pluripotent ICMs, EC cells isolated from teratocarcinomas frequently have abnormal chro-

mosomes and their ability to differentiate is restricted[3,4]. Nevertheless, studies using EC cells have provided valuable information on culture conditions and characterization criteria of pluripotent stem cells.

The strategies used to create normal pluripotent stem cells were very simple. Most important was developing methods to isolate ICM from blastocysts and to maintain the isolated pluripotent stem cells *in vitro*. Mouse embryonic stem (ES) cells from ICM of blastocysts were successfully maintained in a proliferative and undifferentiated state in serum-containing medium on a mouse embryonic fibroblast (MEF) feeder cell layer[5,6]. In addition, leukemia inhibitory factor (LIF) was identified as a protein secreted by the feeder cells that was required to maintain mouse ES cells so that they did not differentiate spontaneously[7,8]. Subsequently, monkey and human ES cells were established under appropriate culture conditions, which differed from those for mouse ES cells because of no effect of LIF on maintenance of the undifferentiated state of these primate cells[9-11]. Human ES cells are cultured in the presence of basic fibroblast growth factor (FGF-2) instead of LIF[12]. Human ES cells as normal pluripotent stem cells provide not only an effective tool to uncover novel biological knowledge related to processes of cell differentiation, but may be stable sources of donor cells for cell-based therapies. Despite these biological advantages of human ES cells, they involve enormous ethical and legal issues due to the destruction of human embryos with potential to develop into human beings.

The establishment of induced-pluripotent stem (iPS) cells has overcome the ethical problems involved in using human ES cells, as well as increasing the applications of pluripotent stem cells. For example, iPS cells established from a patient, who has already been affected by a disease, can be used to analyze the progression of that disease[13-15]. Although iPS cells are associated with several specific problems, including their reduced efficiency of reprogramming, the integration of exogenous DNA into the host genome and the carcinogenic effects of the DNA, these problems may be overcome by various technical improvements[16-20]. In future, pluripotent stem cells, including iPS cells and somatic cell nuclear transfer derived ES cells, will be characterized by comparison to ES cells as the gold standard and will be utilized in many aspects of basic and clinical research, depending on their features[21,22].

NEURONAL DIFFERENTIATION OF PLURIPOTENT STEM CELLS

Stemness, an essential characteristic of a stem cell, involves properties of self-renewal and the potential to differentiate into functional somatic cells. Pluripotent stem cells, like ES and iPS cells, can proliferate infinitely in an undifferentiated state and have the potential to differentiate into any somatic cell derived from the three embryonic germ layers. In contrast, neural stem (NS) cells, defined as stem cells committed to the neural cell lineage, have lost pluripotency and acquired multipotency, or a limited ability to differentiate into several cell types. For example, NS cells can differentiate into neural cells, such as neurons, astrocytes and oligodendrocytes. The pluripotency of cells can be experimentally analyzed by two general methods, teratoma formation *in vivo* and embryoid body (EB) formation *in vitro*[23-25]. In the EB formation method, enzymatically digested mouse ES cells are grown in hanging drop culture in serum-containing media without LIF. These dissociated ES cells immediately form unorganized aggregates, resulting in EBs after several days. These EBs consist of endodermal, mesodermal and ectodermal cells, thus closely resembling early post-implantation embryos[26]. Many attempts have been made to modify this method to improve the reproducibility and efficiency of EB formation[27-29]. Some modifications alter the direction of differentiation *via* EBs, indicating that optimization of culture conditions to form EBs would efficiently bias the direction of differentiation, enabling the preparation of large numbers of desired specialized cells from pluripotent stem cells.

The criteria used to assess differentiation methods include the simplicity of the procedure, the efficiency of differentiation and versatility across animal species. Several methods of neural differentiation have been developed. EB formation is the method used most frequently to assess pluripotency, as described above. However, neural differentiation *via* EB formation is spatiotemporally unusual and not unidirectional, reducing the effective generation of neural cells. To overcome these limitations, retinoic acid, a well-known morphogenic factor, is added to culture media to promote neural differentiation[30]. In addition, FGF-2 may be used to promote the selective proliferation of NS cells from EBs, increasing the total number of NS cells[31]. Unfortunately, even these optimized protocols involve elaborate and time-consuming procedures to generate homogeneous populations of neural cells.

The serum-free cell suspension method is based on EB formation using chemically defined media and secreted factors, similar to those utilized for neurogenesis in embryos[32]. In brief, treatment with Wnt and Nodal antagonists during the formation of EBs promotes the selective differentiation of dissociated mouse ES cells into neural cells. This method, in combination with cell sorting techniques, can efficiently generate central nervous system (CNS) cells, including telencephalic progenitors, retinal progenitors, photoreceptor cells and hypothalamic neurons[32-34]. Another method, dual-SMAD inhibition protocol, is based on monolayer culture with SMAD signaling inhibitors such as noggin and SB431542, generating not only CNS cells like primitive and definitive NS cells, but also neural crest cells from human ES cells with high efficiency[35-37]. In the case of neurogenesis of human ES cells, these methods require application of Rho-associated kinase (ROCK) inhibitor Y-27632 to improve the poor survival of human ES cells after enzymatic

dissociation[32,35]. Recently, it has been reported that this ROCK inhibitor itself promotes neuronal differentiation of mouse ES cells, suggesting that ROCK inhibitor may promote both cell viability after dissociation and improve efficiency of neuronal differentiation of human ES cells[38]. In contrast, these methods based on chemically defined media depend on ready-to-use products, reducing efforts to introduce these experimental methods, For example, the compositions of well-known supplements, including Knockout Serum Replacement and B-27 supplement, have been kept confidential, blocking the ability to prepare and optimize them for use in individual laboratories. In addition, these commercially available supplements vary widely in their ability to support neurons in culture[39]. Lot-to-lot variations in these products should be monitored when using these products in neuroscience research.

UNI-DIRECTIONAL NEURONAL DIFFERENTIATION OF ES CELLS BY THE NEURAL STEM SPHERE METHOD

The neural stem sphere (NSS) method is a simple neural differentiation method using only astrocyte-conditioned medium (ACM) prepared from serum-free medium under free floating conditions[40-42]. In brief, ES cell colonies formed on MEF feeder layers at clonal density are mechanically picked. In the absence of proteolytic digestion, these ES cell colonies maintain a compact shape, like ICM in blastocysts. These ES cell colonies are subsequently cultivated in ACM on bacteriological dishes for short periods of time. Cultivation of rodent and primate ES cell colonies for 4 and 12 d, respectively, results in the efficient development of cell spheres, designated NSSs, which mainly contain NS cells and neurons.

In addition to the ease of performance of this procedure and, its versatility across animal species, the NSS method has some characteristic properties. This method promotes the unidirectional neuronal differentiation of mouse ES cells through stepwise progression, characterized as the synchronous conversion of ES into NS cells through epiblasts as intermediates[43]. The temporal course of this process is comparable to that of neural tube organization from blastocysts during early embryogenesis. Supplementation of ACM with epidermal growth factor (EGF) and FGF-2 accelerates both the proliferation of NS cells and the suppression of neuronal differentiation, resulting in the generation of NSSs composed of a population rich in NS cells, even during the same culture period. Furthermore, adhesion culture of these NS cell-rich NSSs with mitogens, EGF and/or FGF-2 on matrigel-coated tissue culture dishes provides large numbers of homogenous NS cells. These NS cells can be maintained on monolayer cultures with mitogens, can be preserved by freezing, and can differentiate into neurons and glia[44]. Altogether, these findings suggest that the NSS method will provide a platform for considerable biological research on neurodevelopmental processes, including the generation of neuroepithelial cells from pluripotent stem cells, postmitotic neural maturation and neural cell death.

BASIC RESEARCH AND APPLICATIONS USING NEURAL STEM SPHERES AND HOMOGENEOUS NEURALS CELLS

As described above, cell spheres formed using the NSS method mimic neural tissues during early embryogenesis, with NSSs providing homogeneous NS cells that can be maintained on monolayer cultures. Since the platform based on the NSS method will provide novel findings in many biological disciplines, several basic and applied research findings using this platform are described below.

Neural stem sphere as an in vitro model to analyze early neurodevelopment

Understanding the molecular basis underlying early neurogenesis enhances the efficiency of production of neural cells *in vitro*, as well as providing insights into the mechanisms underlying neurodevelopmental disorders. In particular, some information is available about the molecular events associated with the transition from primate ES to neural cells. A search for proteins involved in mouse and monkey neurogenesis from ES cells to NS cells and neurons using two-dimensional gel electrophoresis and peptide mass fingerprinting and NSSs as *in vitro* models have identified seven proteins in mouse and 34 in monkey, all of which specifically change during neuronal differentiation[45-47]. In these proteomic analyses, galectin-1 is identified as a protein which transiently expresses in NS cells during neuronal differentiation of mouse ES cells. This protein is well known to interact with extracellular matrix including laminin and fibronectin, and is involved in neuronal path-finding, neurite outgrowth and axon fasciculation[48-50]. Interestingly, the expression of galectin-1 protein does not change during the conversion of monkey ES cells to neural cells, which is reminiscent of the differences in the mechanisms of neural differentiation of mouse and monkey ES cells. Taken together, these results provide valuable insights into the molecular basis of differentiation and provide novel molecular markers to assess neural cell types during early neurogenesis.

Highly pure and homogeneous, cell populations would likely improve signal-to-noise ratio, resulting in a reliable determination of molecular functions. Although neurospheres derived from neural tissues involve NS cells amplified *in vitro* and maintain the spatiotemporal specific identities of the original tissues, cell populations of the neurospheres are likely to be heterogeneous[51]. In contrast, neural differentiation protocols realize highly pure cell populations of neural cells, particularly NS cells as described above. The expression patterns of genes encoding three BMP/RA-inducible neural-specific pro-

teins (BRINPs) have been assayed during neuronal differentiation of mouse ES cells by the NSS method to determine the functions of these genes associated with the cell-cycle regulation of NS cells[52]. While any *BRINP* genes, *BRINP1, 2* and *3*, express in mouse ES cells with no significant difference, *BRINP1* and *2* highly express in the mouse NSS-derived NS cells. Besides, the *BRINPs* are able to suppress cell cycle progression in NS cells. In a further study, using *BRINP1* knockout mice to clarify the physiological functions of this protein in the CNS, the absence of BRINP1 caused the deregulation of neurogenesis and impaired neuronal differentiation in the adult hippocampal circuitry[53].

Neural stem sphere-derived homogenous neural stem cells for biological research

The self-renewal and multipotency of NS cells are restricted dramatically as neurogenesis progresses *in vivo*[54]. During early neurodevelopmental stages, most NS cells divide symmetrically, generating indistinguishable daughter cells. This proliferation under strict spatiotemporal control declines rapidly, and NS cells gradually produce neurons and glia by asymmetric cell divisions. However, NS cells isolated from embryonic tissue samples may not be stably handled *in vitro*, making it difficult to analyze their properties associated with "stemness" *in vitro*. In contrast, NS cells prepared from ES cells *via* the formation of NSSs stably proliferate without neural differentiation on an adhesive substrate with growth factors[47]. Using these homogeneous mouse NS cells, we have examined the effects of the mitogens, FGF-2 and EGF[55]. Culture with these mitogens enhances the proliferation of NS cells in dose-dependent manners. Subculture of the cells at least five times does not reduce the potential of these cells to self-renew or their multipotency. These results suggest that NS cells prepared from ES cells can actively proliferate under culture conditions containing FGF-2 or EGF. In addition, these homogeneous mouse NS cells can be differentiated almost exclusively into astrocytes solely by withdrawing growth factors from the medium, and that astrocytogenesis occurs through a default pathway[56].

Physical stimuli, including X-irradiation, heat shock, stretch and hypoxia, induce differentiation, proliferation and apoptosis at cellular levels, causing pathogenesis in the CNS *via* ectopic neural differentiation and the degeneration of neural cells *in vivo*[57-62]. However, the complexities of CNS make it difficult to determine whether these effects are directly due to physical stimulation. We have previously investigated the responses of mouse NS cells to X-irradiation, which causes congenital brain abnormalities[63]. Homogeneous NS cells irradiated with X-rays at a dose of 1 Gy maintain the capacity to proliferate and differentiate, although proliferation arrests temporarily. In contrast, cells cease proliferation following irradiation with > 5 Gy, suggesting that irradiation of the fetal brain at relatively low doses may cause congenital brain abnormalities, as does irradiation at relatively high doses.

Hyperthermia during pregnancy is a significant cause of reproductive problems, ranging from abortion to congenital defects of the CNS, including neural tube defects and microcephaly. We have tested the effects of heat shock on homogeneous proliferating mouse NS cells[64]. After heat shock at 42 ℃ for 20 min, the NS cells show stable proliferation, with few changes in gene expression and cell survival and proliferation. In contrast, heat shock at 43 ℃ causes a variety of responses, including the up-regulation of genes encoding heat shock proteins, induction of apoptosis, temporal inhibition of cell proliferation and retardation of neural differentiation. Finally, heat shock at 44 ℃ results in severe effects, with almost all cells disappearing and the remaining cells losing the capacity to proliferate and differentiate. These temperature-dependent effects of heat shock on NS cells may provide insight into the mechanisms by which hyperthermia during pregnancy causes various reproductive problems.

Application of homogeneous neural stem cells to cell transplantation therapies

Primate ES cells have the potential to differentiate into various functional neurons, suggesting that these cells may provide donor cells for cell transplantation therapies in patients with incurable neurodegenerative disorders. Cells are transplanted into patients with Parkinson's disease (PD) primarily for their ability to secrete dopaminergic neurotransmitters into the putamen without functional neural circuits. In one clinical application of stem cells, NS cells derived from cynomolgus ES cells have been implanted unilaterally into the putamen of two cynomolgus monkeys with chronic PD, generated by systemic administration of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine[65]. Positron emission tomography (PET) reveals significantly increased uptake of PET multitracers, isotope-labeled L-DOPA and β-CFT in the grafted putamen, demonstrating that transplantation of NS cells derived from cynomolgus monkey ES cells can restore DA function in the putamen of a primate model of PD.

In addition to PD, spinal cord injury (SCI) is another degenerative disorder which cannot be rectified by current therapies to the extent desired by patients suffering from devastating traumata. To develop a novel radical cure for SCI, astrocytes generated from mouse iPS cell-derived NSSs have been transplanted into the lesions of injured rat spinal cords[66]. Transplant recipients lived for 8 wk without tumor formation. Although locomotive tests demonstrated no improvement compared with control rats, the cell-transplantation led to greater sensitivity to mechanical stimuli. Taken together, these results partially allay a safety concern regarding tumor formation from the transplanted astrocytes, and emphasize the need to determine optimal conditions for the transplantation, *e.g.,* type of neural cell and homogeneity of transplanted cell population.

CONCLUSION

The NSS method is a simple protocol for inducing the

unidirectional neuronal differentiation of pluripotent stem cells by using astrocyte-conditioned medium prepared from serum-free medium. Analyzing the process of this neuronal differentiation can increase the opportunity to explore novel findings during early neurogenesis. These findings deepen the understanding of both the sophisticated mechanisms underlying neurogenesis and the biological variations in neural cells among animals. This, in turn, may provide insights enabling the determination of the cellular etiologies of neurodegenerative disorders and neuropsychiatric diseases. Well-characterized and homogeneous NS cells prepared by the NSS method may act as donor cells for cell transplantation therapies. In addition, the powerful platform based on NSS method will be utilized in a high-throughput, cell fate assay system to assess the effects of innumerable chemical compounds and physical stimuli suspected of being teratogens. This may result in a potentially safer environment in the near future.

REFERENCES

1 **Jewett MA**. Biology of testicular tumors. *Urol Clin North Am* 1977; **4**: 495-507 [PMID: 76359]
2 **Jewett MA**. Testis carcinoma: transplantation into nude mice. *Natl Cancer Inst Monogr* 1978; **49:** 65-66 [PMID: 748798]
3 **Sekiya S**, Kawata M, Iwasawa H, Inaba N, Sugita M, Suzuki N, Motoyama T, Yamamoto T, Takamizawa H. Characterization of human embryonal carcinoma cell lines derived from testicular germ-cell tumors. *Differentiation* 1985; **29**: 259-267 [PMID: 2416623 DOI: 10.1111/j.1432-0436.1985.tb00325.x]
4 **Andrews PW**, Damjanov I, Simon D, Banting GS, Carlin C, Dracopoli NC, Føgh J. Pluripotent embryonal carcinoma clones derived from the human teratocarcinoma cell line Tera-2. Differentiation in vivo and in vitro. *Lab Invest* 1984; **50**: 147-162 [PMID: 6694356]
5 **Evans MJ**, Kaufman MH. Establishment in culture of pluripotential cells from mouse embryos. *Nature* 1981; **292**: 154-156 [PMID: 7242681 DOI: 10.1038/292154a0]
6 **Martin GR**. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. *Proc Natl Acad Sci USA* 1981; **78**: 7634-7638 [PMID: 6950406 DOI: 10.1073/pnas.78.12.7634]
7 **Williams RL**, Hilton DJ, Pease S, Willson TA, Stewart CL, Gearing DP, Wagner EF, Metcalf D, Nicola NA, Gough NM. Myeloid leukaemia inhibitory factor maintains the developmental potential of embryonic stem cells. *Nature* 1988; **336**: 684-687 [PMID: 3143916 DOI: 10.1038/336684a0]
8 **Niwa H**, Burdon T, Chambers I, Smith A. Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. *Genes Dev* 1998; **12**: 2048-2060 [PMID: 9649508 DOI: 10.1101/gad.12.13.2048]
9 **Thomson JA**, Kalishman J, Golos TG, Durning M, Harris CP, Becker RA, Hearn JP. Isolation of a primate embryonic stem cell line. *Proc Natl Acad Sci USA* 1995; **92**: 7844-7848 [PMID: 7544005 DOI: 10.1073/pnas.92.17.7844]
10 **Thomson JA**, Kalishman J, Golos TG, Durning M, Harris CP, Hearn JP. Pluripotent cell lines derived from common marmoset (Callithrix jacchus) blastocysts. *Biol Reprod* 1996; **55**: 254-259 [PMID: 8828827 DOI: 10.1095/biolreprod55.2.254]
11 **Thomson JA**, Itskovitz-Eldor J, Shapiro SS, Waknitz MA, Swiergiel JJ, Marshall VS, Jones JM. Embryonic stem cell lines derived from human blastocysts. *Science* 1998; **282**: 1145-1147 [PMID: 9804556 DOI: 10.1126/science.282.5391.1145]
12 **Levenstein ME**, Ludwig TE, Xu RH, Llanas RA, VanDenHeuvel-Kramer K, Manning D, Thomson JA. Basic fibroblast growth factor support of human embryonic stem cell self-renewal. *Stem Cells* 2006; **24**: 568-574 [PMID: 16282444 DOI: 10.1634/stemcells.2005-0247]
13 **Takahashi K**, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 2006; **126**: 663-676 [PMID: 16904174 DOI: 10.1016/j.cell.2006.07.024]
14 **Takahashi K**, Tanabe K, Ohnuki M, Narita M, Ichisaka T, Tomoda K, Yamanaka S. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell* 2007; **131**: 861-872 [PMID: 18035408 DOI: 10.1016/j.cell.2007.11.019]
15 **Yu J**, Vodyanik MA, Smuga-Otto K, Antosiewicz-Bourget J, Frane JL, Tian S, Nie J, Jonsdottir GA, Ruotti V, Stewart R, Slukvin II, Thomson JA. Induced pluripotent stem cell lines derived from human somatic cells. *Science* 2007; **318**: 1917-1920 [PMID: 18029452 DOI: 10.1126/science.1151526]
16 **Okita K**, Matsumura Y, Sato Y, Okada A, Morizane A, Okamoto S, Hong H, Nakagawa M, Tanabe K, Tezuka K, Shibata T, Kunisada T, Takahashi M, Takahashi J, Saji H, Yamanaka S. A more efficient method to generate integration-free human iPS cells. *Nat Methods* 2011; **8**: 409-412 [PMID: 21460823 DOI: 10.1038/nmeth.1591]
17 **Nishishita N**, Takenaka C, Fusaki N, Kawamata S. Generation of human induced pluripotent stem cells from cord blood cells. *J Stem Cells* 2011; **6**: 101-108 [PMID: 23264996]
18 **Stadtfeld M**, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. *Science* 2008; **322**: 945-949 [PMID: 18818365 DOI: 10.1126/science.1162494]
19 **Warren L**, Manos PD, Ahfeldt T, Loh YH, Li H, Lau F, Ebina W, Mandal PK, Smith ZD, Meissner A, Daley GQ, Brack AS, Collins JJ, Cowan C, Schlaeger TM, Rossi DJ. Highly efficient reprogramming to pluripotency and directed differentiation of human cells with synthetic modified mRNA. *Cell Stem Cell* 2010; **7**: 618-630 [PMID: 20888316 DOI: 10.1016/j.stem.2010.08.012]
20 **Kim D**, Kim CH, Moon JI, Chung YG, Chang MY, Han BS, Ko S, Yang E, Cha KY, Lanza R, Kim KS. Generation of human induced pluripotent stem cells by direct delivery of reprogramming proteins. *Cell Stem Cell* 2009; **4**: 472-476 [PMID: 19481515 DOI: 10.1016/j.stem.2009.05.005]
21 **Tachibana M**, Amato P, Sparman M, Gutierrez NM, Tippner-Hedges R, Ma H, Kang E, Fulati A, Lee HS, Sritanaudomchai H, Masterson K, Larson J, Eaton D, Sadler-Fredd K, Battaglia D, Lee D, Wu D, Jensen J, Patton P, Gokhale S, Stouffer RL, Wolf D, Mitalipov S. Human embryonic stem cells derived by somatic cell nuclear transfer. *Cell* 2013; **153**: 1228-1238 [PMID: 23683578 DOI: 10.1016/j.cell.2013.05.006]
22 **Yamanaka S**. Induced pluripotent stem cells: past, present, and future. *Cell Stem Cell* 2012; **10**: 678-684 [PMID: 22704507 DOI: 10.1016/j.stem.2012.05.005]
23 **Kleinsmith LJ,** Pierce GB. Multipotentiality of single embryonal carcinoma cells. *Cancer Res* 1964; **24**: 1544-1551 [PMID: 14234000]
24 **Martin GR**, Evans MJ. Differentiation of clonal lines of teratocarcinoma cells: formation of embryoid bodies in vitro. *Proc Natl Acad Sci USA* 1975; **72**: 1441-1445 [PMID: 1055416 DOI: 10.1073/pnas.72.4.1441]
25 **Guan K**, Czyz J, Fürst DO, Wobus AM. Expression and cellular distribution of alpha(v)integrins in beta(1)integrin-deficient embryonic stem cell-derived cardiac cells. *J Mol Cell Cardiol* 2001; **33**: 521-532 [PMID: 11181020 DOI: 10.1006/jmcc.2000.1326]
26 **O'Shea KS**. Embryonic stem cell models of development. *Anat Rec* 1999; **257**: 32-41 [PMID: 10333401 DOI: 10.1002/(SICI)1097-0185(19990215)257: 1<32: : AID-AR6>3.0.CO; 2-2]
27 **Gökhan S**, Mehler MF. Basic and clinical neuroscience applications of embryonic stem cells. *Anat Rec* 2001; **265**:

142-156 [PMID: 11458329 DOI: 10.1002/ar.1136]
28 **Dang SM**, Kyba M, Perlingeiro R, Daley GQ, Zandstra PW. Efficiency of embryoid body formation and hematopoietic development from embryonic stem cells in different culture systems. *Biotechnol Bioeng* 2002; **78**: 442-453 [PMID: 11948451 DOI: 10.1002/bit.10220]
29 **Serra M**, Brito C, Costa EM, Sousa MF, Alves PM. Integrating human stem cell expansion and neuronal differentiation in bioreactors. *BMC Biotechnol* 2009; **9**: 82 [PMID: 19772662 DOI: 10.1186/1472-6750-9-82]
30 **Bain G**, Kitchens D, Yao M, Huettner JE, Gottlieb DI. Embryonic stem cells express neuronal properties in vitro. *Dev Biol* 1995; **168**: 342-357 [PMID: 7729574 DOI: 10.1006/dbio.1995.1085]
31 **Okabe S**, Forsberg-Nilsson K, Spiro AC, Segal M, McKay RD. Development of neuronal precursor cells and functional postmitotic neurons from embryonic stem cells in vitro. *Mech Dev* 1996; **59**: 89-102 [PMID: 8892235 DOI: 10.1016/0925-4773(96)00572-2]
32 **Watanabe K**, Kamiya D, Nishiyama A, Katayama T, Nozaki S, Kawasaki H, Watanabe Y, Mizuseki K, Sasai Y. Directed differentiation of telencephalic precursors from embryonic stem cells. *Nat Neurosci* 2005; **8**: 288-296 [PMID: 15696161 DOI: 10.1038/nn1402]
33 **Ikeda H**, Osakada F, Watanabe K, Mizuseki K, Haraguchi T, Miyoshi H, Kamiya D, Honda Y, Sasai N, Yoshimura N, Takahashi M, Sasai Y. Generation of Rx+/Pax6+ neural retinal precursors from embryonic stem cells. *Proc Natl Acad Sci USA* 2005; **102**: 11331-11336 [PMID: 16076961 DOI: 10.1073/pnas.0500010102]
34 **Suga H**, Kadoshima T, Minaguchi M, Ohgushi M, Soen M, Nakano T, Takata N, Wataya T, Muguruma K, Miyoshi H, Yonemura S, Oiso Y, Sasai Y. Self-formation of functional adenohypophysis in three-dimensional culture. *Nature* 2011; **480**: 57-62 [PMID: 22080957 DOI: 10.1038/nature10637]
35 **Chambers SM**, Fasano CA, Papapetrou EP, Tomishima M, Sadelain M, Studer L. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. *Nat Biotechnol* 2009; **27**: 275-280 [PMID: 19252484 DOI: 10.1038/nbt.1529]
36 **Mica Y**, Lee G, Chambers SM, Tomishima MJ, Studer L. Modeling neural crest induction, melanocyte specification, and disease-related pigmentation defects in hESCs and patient-specific iPSCs. *Cell Rep* 2013; **3**: 1140-1152 [PMID: 23583175 DOI: 10.1016/j.celrep.2013.03.025]
37 **Rodrigues GM**, Matos AF, Fernandes TG, Rodrigues CA, Peitz M, Haupt S, Diogo MM, Brüstle O, Cabral JM. Integrated platform for production and purification of human pluripotent stem cell-derived neural precursors. *Stem Cell Rev* 2014; **10**: 151-161 [PMID: 24221956 DOI: 10.1007/s12015-013-9482-z]
38 **Kamishibahara Y**, Kawaguchi H, Shimizu N. Promotion of mouse embryonic stem cell differentiation by Rho kinase inhibitor Y-27632. *Neurosci Lett* 2014; **579**: 58-63 [PMID: 25038419 DOI: 10.1016/j.neulet.2014.07.011]
39 **Chen Y**, Stevens B, Chang J, Milbrandt J, Barres BA, Hell JW. NS21: re-defined and modified supplement B27 for neuronal cultures. *J Neurosci Methods* 2008; **171**: 239-247 [PMID: 18471889 DOI: 10.1016/j.jneumeth.2008.03.013]
40 **Nakayama T**, Momoki-Soga T, Inoue N. Astrocyte-derived factors instruct differentiation of embryonic stem cells into neurons. *Neurosci Res* 2003; **46**: 241-249 [PMID: 12767487 DOI: 10.1016/S0168-0102(03)00063-4]
41 **Nakayama T**, Inoue N. Neural stem sphere method: induction of neural stem cells and neurons by astrocyte-derived factors in embryonic stem cells in vitro. *Methods Mol Biol* 2006; **330**: 1-13 [PMID: 16846013]
42 **Okuno T**, Nakayama T, Konishi N, Michibata H, Wakimoto K, Suzuki Y, Nito S, Inaba T, Nakano I, Muramatsu S, Takano M, Kondo Y, Inoue N. Self-contained induction of neurons from human embryonic stem cells. *PLoS One* 2009; **4**: e6318 [PMID: 19621077 DOI: 10.1371/journal.pone.0006318]
43 **Otsu M**, Sai T, Nakayama T, Murakami K, Inoue N. Unidirectional differentiation of mouse embryonic stem cells into neurons by the neural stem sphere method. *Neurosci Res* 2011; **69**: 314-321 [PMID: 21192990 DOI: 10.1016/j.neures.2010.12.014]
44 **Nakayama T**, Momoki-Soga T, Yamaguchi K, Inoue N. Efficient production of neural stem cells and neurons from embryonic stem cells. *Neuroreport* 2004; **15**: 487-491 [PMID: 15094509 DOI: 10.1097/00001756-200403010-00021]
45 **Akama K**, Tatsuno R, Otsu M, Horikoshi T, Nakayama T, Nakamura M, Toda T, Inoue N. Proteomic identification of differentially expressed genes in mouse neural stem cells and neurons differentiated from embryonic stem cells in vitro. *Biochim Biophys Acta* 2008; **1784**: 773-782 [PMID: 18328832 DOI: 10.1016/j.bbapap.2008.02.001]
46 **Akama K**, Horikoshi T, Nakayama T, Otsu M, Imaizumi N, Nakamura M, Toda T, Inuma M, Hirano H, Kondo Y, Suzuki Y, Inoue N. Proteomic identification of differentially expressed genes in neural stem cells and neurons differentiated from embryonic stem cells of cynomolgus monkey (Macaca fascicularis) in vitro. *Biochim Biophys Acta* 2011; **1814**: 265-276 [PMID: 21047566 DOI: 10.1016/j.bbapap.2010.10.009]
47 **Akama K**, Horikoshi T, Nakayama T, Otsu M, Imaizumi N, Nakamura M, Toda T, Inuma M, Hirano H, Kondo Y, Suzuki Y, Inoue N. Proteomic identification of differentially expressed genes during differentiation of cynomolgus monkey (Macaca fascicularis) embryonic stem cells to astrocyte progenitor cells in vitro. *Biochim Biophys Acta* 2013; **1834**: 601-610 [PMID: 23232153 DOI: 10.1016/j.bbapap.2012.12.002]
48 **Hughes RC**. Galectins as modulators of cell adhesion. *Biochimie* 2001; **83**: 667-676 [PMID: 11522396 DOI: 10.1016/S0300-9084(01)01289-5]
49 **Mahanthappa NK**, Cooper DN, Barondes SH, Schwarting GA. Rat olfactory neurons can utilize the endogenous lectin, L-14, in a novel adhesion mechanism. *Development* 1994; **120**: 1373-1384 [PMID: 8050350]
50 **Puche AC**, Poirier F, Hair M, Bartlett PF, Key B. Role of galectin-1 in the developing mouse olfactory system. *Dev Biol* 1996; **179**: 274-287 [PMID: 8873770 DOI: 10.1006/dbio.1996.0257]
51 **Parker MA**, Anderson JK, Corliss DA, Abraria VE, Sidman RL, Park KI, Teng YD, Cotanche DA, Snyder EY. Expression profile of an operationally-defined neural stem cell clone. *Exp Neurol* 2005; **194**: 320-332 [PMID: 15992799 DOI: 10.1016/j.expneurol.2005.04.018]
52 **Terashima M**, Kobayashi M, Motomiya M, Inoue N, Yoshida T, Okano H, Iwasaki N, Minami A, Matsuoka I. Analysis of the expression and function of BRINP family genes during neuronal differentiation in mouse embryonic stem cell-derived neural stem cells. *J Neurosci Res* 2010; **88**: 1387-1393 [PMID: 20025061 DOI: 10.1002/jnr.22315]
53 **Kobayashi M**, Nakatani T, Koda T, Matsumoto K, Ozaki R, Mochida N, Takao K, Miyakawa T, Matsuoka I. Absence of BRINP1 in mice causes increase of hippocampal neurogenesis and behavioral alterations relevant to human psychiatric disorders. *Mol Brain* 2014; **7**: 12 [PMID: 24528488 DOI: 10.1186/1756-6606-7-12]
54 **Temple S**. The development of neural stem cells. *Nature* 2001; **414**: 112-117 [PMID: 11689956 DOI: 10.1038/35102174]
55 **Yoshie T,** Omori H, Otsu M, Shibata M, Nakayama T, Inoue N. Effects of mitogens on mouse embryonic stem cell-derived neural stem cells. *J Jpn Health Sci* 2014; **16:** 201-209
56 **Nakayama T**, Sai T, Otsu M, Momoki-Soga T, Inoue N. Astrocytogenesis of embryonic stem-cell-derived neural stem cells: Default differentiation. *Neuroreport* 2006; **17**: 1519-1523 [PMID: 16957601]

57 **Amano T**, Inamura T, Wu CM, Kura S, Nakamizo A, Inoha S, Miyazono M, Ikezaki K. Effects of single low dose irradiation on subventricular zone cells in juvenile rat brain. *Neurol Res* 2002; **24**: 809-816 [PMID: 12500705 DOI: 10.1179/016164102101200771]

58 **Afzal E**, Ebrahimi M, Najafi SM, Daryadel A, Baharvand H. Potential role of heat shock proteins in neural differentiation of murine embryonal carcinoma stem cells (P19). *Cell Biol Int* 2011; **35**: 713-720 [PMID: 21355853 DOI: 10.1042/CBI20100457]

59 **von Reyn CR**, Mott RE, Siman R, Smith DH, Meaney DF. Mechanisms of calpain mediated proteolysis of voltage gated sodium channel α-subunits following in vitro dynamic stretch injury. *J Neurochem* 2012; **121**: 793-805 [PMID: 22428606 DOI: 10.1111/j.1471-4159.2012.07735.x]

60 **Ma M**, Li L, Wang X, Bull DL, Shofer FS, Meaney DF, Neumar RW. Short-duration treatment with the calpain inhibitor MDL-28170 does not protect axonal transport in an in vivo model of traumatic axonal injury. *J Neurotrauma* 2012; **29**: 445-451 [PMID: 22077394 DOI: 10.1089/neu.2011.2060]

61 **Mohyeldin A**, Garzón-Muvdi T, Quiñones-Hinojosa A. Oxygen in stem cell biology: a critical component of the stem cell niche. *Cell Stem Cell* 2010; **7**: 150-161 [PMID: 20682444 DOI: 10.1016/j.stem.2010.07.007]

62 **Kichev A**, Rousset CI, Baburamani AA, Levison SW, Wood TL, Gressens P, Thornton C, Hagberg H. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) signaling and cell death in the immature central nervous system after hypoxia-ischemia and inflammation. *J Biol Chem* 2014; **289**: 9430-9439 [PMID: 24509861 DOI: 10.1074/jbc.M113.512350]

63 **Isono M**, Otsu M, Konishi T, Matsubara K, Tanabe T, Nakayama T, Inoue N. Proliferation and differentiation of neural stem cells irradiated with X-rays in logarithmic growth phase. *Neurosci Res* 2012; **73**: 263-268 [PMID: 22561132 DOI: 10.1016/j.neures.2012.04.005]

64 **Omori H**, Otsu M, Suzuki A, Nakayama T, Akama K, Watanabe M, Inoue N. Effects of heat shock on survival, proliferation and differentiation of mouse neural stem cells. *Neurosci Res* 2014; **79**: 13-21 [PMID: 24316183 DOI: 10.1016/j.neures.2013.11.005]

65 **Muramatsu S**, Okuno T, Suzuki Y, Nakayama T, Kakiuchi T, Takino N, Iida A, Ono F, Terao K, Inoue N, Nakano I, Kondo Y, Tsukada H. Multitracer assessment of dopamine function after transplantation of embryonic stem cell-derived neural stem cells in a primate model of Parkinson's disease. *Synapse* 2009; **63**: 541-548 [PMID: 19253400 DOI: 10.1002/syn.20634]

66 **Hayashi K,** Hashimoto M, Koda M, Naito AT, Murata A, Okawa A, Takahashi K, Yamazaki M. Increase of sensitivity to mechanical stimulus after transplantation of murine induced pluripotent stem cell-derived astrocytes in a rat spinal cord injury model. *J Neurosurg Spine* 2011; **15**: 582-593 [PMID: 21854127 DOI: 10.3171/2011.7.SPINE10775]

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