Differentiation ▮ (▮▮▮▮) ▮▮▮–▮▮▮

Contents lists available at ScienceDirect

Differentiation

journal homepage: www.elsevier.com/locate/diff

Review

Embryonic stem cells in non-human primates: An overview of neural differentiation potential

Florence Wianny [a,b,*], Pierre-Yves Bourillot [a,b], Colette Dehay [a,b]

[a] Inserm, U846, Stem Cell and Brain Research Institute, 18 Avenue Doyen Lépine, 69500 Bron, France
[b] Université de Lyon, Lyon 1, UMR-S 846, 69003 Lyon, France

ARTICLE INFO

Article history:
Received 2 November 2010
Received in revised form
18 December 2010
Accepted 11 January 2011

Keywords:
Embryonic stem cells ESC
Non-human Primate
Self-renewal
Neural differentiation
Neural stem cells

ABSTRACT

Non-human primate (NHP) embryonic stem (ES) cells show unlimited proliferative capacities and a great potential to generate multiple cell lineages. These properties make them an ideal resource both for investigating early developmental processes and for assessing their therapeutic potential in numerous models of degenerative diseases. They share the same markers and the same properties with human ES cells, and thus provide an invaluable transitional model that can be used to address the safety issues related to the clinical use of human ES cells. Here, we review the available information on the derivation and the specific features of monkey ES cells. We comment on the capacity of primate ES cells to differentiate into neural lineages and the current protocols to generate self-renewing neural stem cells. We also highlight the signalling pathways involved in the maintenance of these neural cell types. Finally, we discuss the potential of monkey ES cells for neuronal differentiation.

© 2011 International Society of Differentiation. Published by Elsevier Ltd. All rights reserved.

Contents

1. Introduction . . . 2
2. Derivation and specific features of NHP ES cells . . . 2
   2.1. The origin and derivation of monkey ES cell lines . . . 2
   2.2. Surface and molecular markers . . . 2
   2.3. Genetic integrity . . . 2
   2.4. Self-renewal regulation and cell cycle features . . . 3
   2.5. Genetic labelling . . . 4
   2.6. Pluripotency . . . 4
3. Differentiation along neural lineages: the lessons from human ES cells . . . 5
   3.1. Early neural differentiation . . . 5
   3.2. Regional identity . . . 5
   3.3. Isolating multipotent neural stem cell lines . . . 5
   3.4. Signalling pathways involved in neural induction and maintenance of NSCs . . . 6
   3.5. From neural induction to cortical differentiation . . . 7
   3.6. Neuronal differentiation of monkey ES cells and their behaviour *in vivo* . . . 7

Abbreviations: BMP, bone morphogenetic protein; DA, dopaminergic; EB, embryoid body; EC, embryonal carcinoma; ES, embryonic stem; EGF, epidermal growth factor; FBS, foetal bovine serum; FGF2, fibroblast growth factor 2; hES cell, human ES cell; Forse-1, forebrain-surface-embryonic 1; ICM, inner cell mass; lt-ESNSC, long term self-renewing NSC; LIF, leukaemia inhibitory factor; NHP, non-human primate; NSC, neural stem cell; PD, Parkinson's disease; RA, retinoic acid; R-NSC, rosette stage NSC; SSEA, stage specific embryonic antigen; SDIA, stromal cell induced activity; SHH, sonic hedgehog; STAT3, signal transducer and activator of transcription 3; TGFβ, transforming growth factor β

* Corresponding author at: Inserm, U846, Stem Cell and Brain Research Institute, 18 Avenue Doyen Lépine, 69500 Bron, France. Tel.: +33 4 72 91 34 66; fax: +33 4 72 91 34 61.
E-mail addresses: florence.wianny@inserm.fr (F. Wianny), pierre-yves.bourillot@inserm.fr (P.-Y. Bourillot), colette.dehay@inserm.fr (C. Dehay).

0301-4681/$ - see front matter © 2011 International Society of Differentiation. Published by Elsevier Ltd. All rights reserved.
Join the International Society for Differentiation (www.isdifferentiation.org)
doi:10.1016/j.diff.2011.01.008

Please cite this article as: Wianny, F., et al., Embryonic stem cells in non-human primates: An overview of neural differentiation potential. Differentiation (2011), doi:10.1016/j.diff.2011.01.008

ARTICLE IN PRESS

4. Concluding remarks . . . 8
Acknowledgements . . . 8
References . . . 8

1. Introduction

Embryonic stem cells (ES) are pluripotent cells derived from pre-implantation embryos. They have indefinite replication potential and the ability to differentiate into all adult cell types (Evans and Kaufman, 1981). These properties render them an ideal resource for the *in vitro* study of developmental processes, and for transplantation therapy in numerous pathologies (Lerou and Daley, 2005). The successful derivation of ES cells was not straightforward (Hogan and Tilly, 1977). A promising start to derive pluripotent cells from early embryos was made in the early 1970s, when it was found that teratocarcinoma developed after grafting early mouse embryos underneath the kidney capsule or in the testis of adult mice (Solter et al., 1970; Stevens, 1970). These teratocarcinomas contained undifferentiated pluripotent cells, called embryonal carcinoma (EC) cells that could be expanded continuously in culture. EC cells were also able to differentiate either *in vitro* in suspension cultures (Martin and Evans, 1975) or *in vivo*, upon grafting into histocompatible mice, and were even capable to participate in normal embryogenesis after injection into an early embryo (Papaioannou et al., 1975). Unfortunately, the efficiency of chimerism with most EC cell lines was low, and the full developmental capacity could not be attained since these cells did not colonise the germ line. By implementing the same conditions that were used for successful isolation and expansion of EC cells, the first ES cell lines were isolated in the mouse 10 years later by two groups (Evans and Kaufman, 1981; Martin, 1981). More recently, the isolation of ES cells in non-human primates (NHP) in the mid 1990s, just prior to the derivation of ES cell lines in human, has generated tremendous interest because it raised the possibility to develop a relevant preclinical model close to human, and assess the benefit, safety and efficacy of ES-derived cell transplantation technologies. Information gained from the study of NHP ES cells, particularly those regarding cell grafting, are of paramount importance. This review summarises the progress achieved in the field of NHP ES cells since their first isolation in 1995, emphasising on their potential for neural differentiation.

2. Derivation and specific features of NHP ES cells

2.1. The origin and derivation of monkey ES cell lines

ES cells are derived from the inner cell mass (ICM) of pre-implantation stage embryos, which will give rise to all foetal tissues. ES cell lines have been derived from several NHP species. The first lines were isolated in the Rhesus monkey (*Macaca mulatta*) in 1995 by Dr. James Thomson at the Wisconsin National Primate Research Center (Thomson et al., 1995), and few years later in the cynomolgus monkey (*Macaca fascicularis*) (Suemori et al., 2001). Several ES cell lines have since been derived either from *in vivo* (Thomson et al., 1995) or from *in vitro* produced Rhesus embryos (Pau and Wolf, 2004; Wolf et al., 2004; Mitalipov et al., 2006; Byrne et al., 2006; Navara et al., 2007; Wianny et al., 2008), and from Baboon (Simerly et al., 2009) and Common Marmoset embryos (*Callithrix jacchus*, a New world monkey) (Thomson et al., 1996; Sasaki et al., 2005; Muller et al., 2009). Syngeneic monkey ES cell lines have also been isolated from parthenogenetically activated oocytes (Cibelli et al., 2002; Vrana et al., 2003) and recently from embryos obtained after somatic cell nuclear transfer (Byrne et al., 2006).

The techniques used for the isolation of monkey ES cell lines are derived from the mouse model. The ICM, isolated mechanically or by immunosurgery (Nichols and Gardner, 1984; Mitalipov et al., 2006) or alternatively the whole embryo, is plated onto a layer of mitotically inactive mouse embryonic fibroblasts. The proliferating cells from the ICM are picked mechanically and replated on a new layer of feeder cells. They are then expanded until a stable line is safely established. The efficiency of ES cell line derivation in the monkey is highly variable (5–50%) (Suemori et al., 2001; Mitalipov et al., 2006; Wianny et al., 2008), and it was found that increased blastocyst age contributes to a progressively increasing efficiency in ES cell line derivation (Mitalipov et al., 2006). Differences in growth rates were also noted during the establishment and propagation of some Rhesus cell lines. This variability in the efficiency of derivation and growth rates is at present difficult to explain, probably reflecting the quality of the embryo, and the variation in the manipulation techniques/skills during the initial derivation stages, as well as in the culture conditions (Mitalipov et al., 2006). Indeed, the conventional medium used for deriving and expanding ES cell lines contains 15–20% foetal bovine serum (FBS). We, as well as others, found that substituting FBS by serum replacement (KOSR) and Fibroblast Growth Factor 2 (FGF2) improved proliferation rates and increased the homogeneity of monkey ES cell morphology (Suemori et al., 2001; Wianny et al., 2008).

2.2. Surface and molecular markers

Monkey ES cells can routinely be expanded to give homogenous, undifferentiated populations with characteristics remarkably similar to human ES (hES) cells, including morphology, pluripotency and surface marker expression (Fig. 1). They express specific cell surface markers including stage specific embryonic antigen (SSEA)4, the glycoprotein's TRA-1-60, TRA-1-81, CD90, but not SSEA1, in contrast to mouse ES cells (Table 1). Whereas rhesus, marmoset and human ES cells express SSEA3 with variable levels, cynomolgus ES cells do not express SSEA3 (Suemori et al., 2001). Monkey ES cells also show high alkaline phosphatase activity, and express several "stemness" genes including *Oct4*, *Sox2* and *Nanog*. These surface and molecular markers are all rapidly down regulated upon differentiation. Comparing five Rhesus ES cell lines, Mitalipov and collaborators observed that more than 10% of their transcriptomes showed significant changes in gene expression. However, these five ES cell lines consistently and strongly expressed a subset of stem cell marker genes *NANOG*, *LIN-28*, *PODXL*, *POU5F1* and *GDF-3*, suggesting that these genes are key regulators of monkey ES cells (Mitalipov, 2006).

2.3. Genetic integrity

Monkey ES cell lines have a normal karyotype with a diploid set of 42 chromosomes. In contrast to the mouse, where male ES cell lines are preferentially established, male and female lines have been derived with the same efficiency in monkey (Suemori et al., 2001; Mitalipov et al., 2006). Abnormal karyotypes were observed in some lines (Mitalipov et al., 2006). Chromosomal abnormalities have also been described in hES cell lines and have been associated with exposure to an extended feeder-free culture system on Matrigel or with enzymatic passaging (Draper et al., 2004; Maitra et al., 2005;

Please cite this article as: Wianny, F., et al., Embryonic stem cells in non-human primates: An overview of neural differentiation potential. Differentiation (2011), doi:10.1016/j.diff.2011.01.008

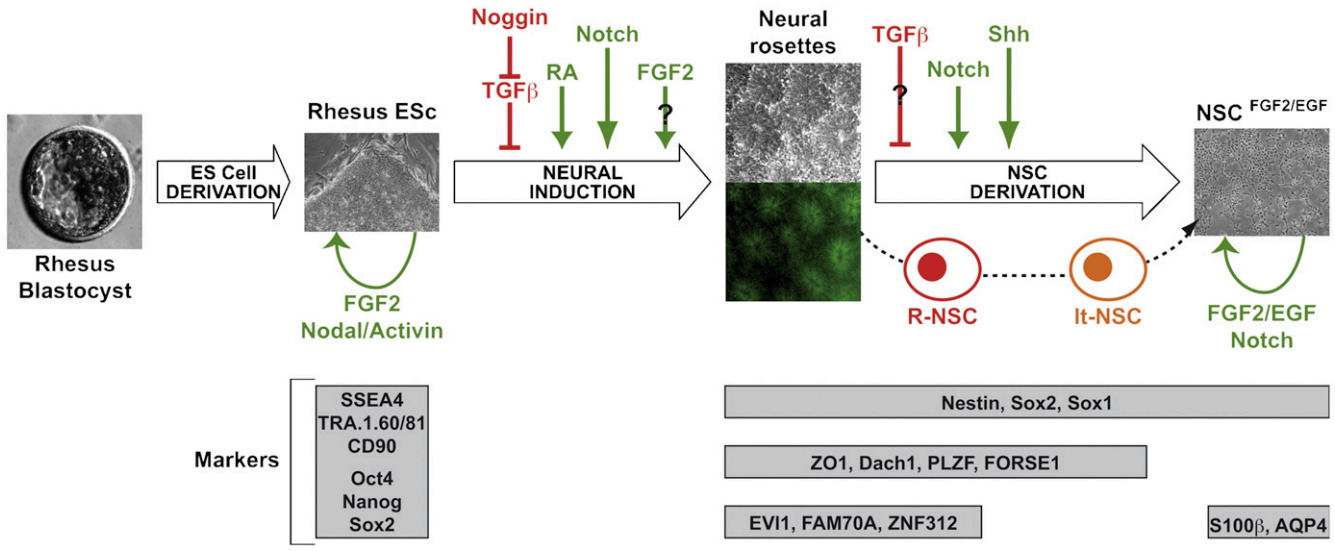

Fig. 1. Isolation of ES cells and neural stem cells in the primate. Schematic representation of the different steps involved in the isolation of ES cells from monkey blastocysts and leading to the derivation of Neural Stem Cells. ES cells can differentiate into neural rosettes: shown in this panel are phase contrast and fluorescent live imaging of radial arrangements of columnar cells expressing TauGFP that nicely labels rosette structures. Neural Stem Cells ($NSCs^{FGF2/EGF}$) can subsequently be derived and amplified in a serum free medium in the presence of EGF and FGF2. R-NSCs and It-ESNSCs are also indicated as intermediate stages during the isolation of NSCs, although they have been so far only isolated in the human. The temporal expression of specific markers is indicated in the grey boxes. Green and red arrows show positive and negative regulatory pathways involved at the different steps, and mainly deciphered in human. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Comparison of mouse, human and monkey ES cells.

Marker	Mouse	Human	Rhesus *Macaca mulatta*	Cynomolgus *Macaca fascicularis*	Baboon *Papio anubis*	Common Marmoset *Callithrix jacchus*
SSEA1	+	–	–	–	–	–
SSEA3	–	+	+	–	?	+
SSEA4	–	+	+	+	Weak	+
TRA-1-60	–[a]	+	+	+	+	+
TRA-1-81	–[a]	+	+	+	+	+
CD90	–	+	+	?	?	?
Alkaline phosphatase	+	+	+	+	?	+
oct4/sox2/nanog	+	+	+	+	+	+
Telomerase activity	High	High	High	High	?	High
Karyotype	40	46	42	42	42	46

?—A question mark indicates that expression is not known.

[a] Antibodies do no react with mouse cells.

Imreh et al., 2006; Werbowetski-Ogilvie et al., 2009). Retention of a normal karyotype thus depends on adherence to scrupulous and stable tissue culture conditions, avoiding any unnecessary stress such as abrupt changes of the culture conditions (temperature, pH, medium composition, overgrowth, etc.). The evaluation of genetic and epigenetic integrity of primate ES cells have been reviewed extensively elsewhere (Byrne et al., 2006; Mitalipov, 2006) and will not be considered further in this review.

2.4. Self-renewal regulation and cell cycle features

Monkey ES cells can be expanded for more than one year (Thomson et al., 1995), while retaining their pluripotent characteristics and by contrast to what has been reported, very large populations of cells can be rapidly generated. Consistent with their long lifespan property, they show high telomerase activity (Sasaki et al., 2005; Wianny et al., 2008; Muller et al., 2009). Like mouse ES cells, they spend more than half of their total cell cycle in S-phase, and exhibit an unusual very short G1 phase (Savatier et al., 2002; Fluckiger et al., 2006). Interestingly, we found that depending on culture conditions, cell-cycle duration of Rhesus ES cells varies between 9 h (when cells are cultured in 20% KO-SR and FGF2) and 15.5 h (when cells are cultured in a medium containing 20% FBS), with 23% of the cells in G1, 58% in S, and 19% in G2/M phases of the cell cycle. HES cells exhibit similar characteristics, with a short cell cycle (15–16 h) and a shortened G1 phase (Becker et al., 2006). Differentiation of Rhesus ES cells is accompanied by a dramatic increase in the fraction of cells in G1 (Fluckiger et al., 2006; Wianny et al., 2008). Interestingly, similar observations have been recently reported in hES cells (Becker et al., 2006, 2010; Filipczyk et al., 2007).

Because of this high proliferation rate, primate ES cells represent an unlimited source of cells for regenerative medicine, provided that unwanted cellular proliferation and tumour formation can be controlled. The control mechanisms of these processes are still poorly understood in monkey ES cells. Much progress has been made to decipher these mechanisms in hES cells, and this is more than probably transposable to monkey ES cells.

In the mouse, ES cells can easily be propagated in medium supplemented with serum and recombinant Leukaemia Inhibitory

Please cite this article as: Wianny, F., et al., Embryonic stem cells in non-human primates: An overview of neural differentiation potential. Differentiation (2011), doi:10.1016/j.diff.2011.01.008

ARTICLE IN PRESS

Factor (LIF). LIF engages a heterodimeric receptor complex consisting of LIF receptor and gp130. This complex recruits the signal transducer and activator of transcription 3 (STAT3) (Boeuf et al., 1997). Expression of a dominant negative STAT3 mutant in mouse ES cells forces differentiation (Niwa et al., 1998), whereas expression of a hormone-dependent STAT3 mutant that can be activated directly by oestradiol sustains self-renewal without the addition of LIF (Matsuda et al., 1999). These data, and others, demonstrate that the LIF/STAT3 pathway plays a central role in the control of self-renewal and pluripotency of mouse ES cells (Burdon et al., 2002).

Unlike mouse ES cells, the propagation of both monkey and human ES cells is not dependent on LIF-signalling (Thomson et al., 1995; Suemori et al., 2001; Pera and Trounson, 2004; Sumi et al., 2004). Several reports indicate that monkey ES cells (Sumi et al., 2004; Sasaki et al., 2005) and hES cells (Daheron et al., 2004; Humphrey et al., 2004) express components of the LIF/STAT3 signalling. STAT3 is phosphorylated in monkey and human ES cells after stimulation with LIF (Daheron et al., 2004; Humphrey et al., 2004; Sumi et al., 2004). Immunohistochemical detection of STAT3 shows that LIF induces STAT3 nuclear translocation in monkey ES cells (Sumi et al., 2004). However, expression of a constitutively active STAT3 mutant fails to sustain self-renewal of hES cells in the absence of feeder cells or conditioned medium (Daheron et al., 2004). In addition, expression of a dominant negative mutant of STAT3 in monkey ES cells does not induce their differentiation (Sumi et al., 2004). Together these data indicate that both monkey and human ES cells are able to activate the LIF/gp130/STAT3 pathway, but this activation does not inhibit their differentiation. Nevertheless, phosphorylation of STAT3 on Ser 727, which is required for its full transcriptional activity, is observed in monkey ES cells cultured on feeder cells or in feeder conditioned medium. Phosphorylation of STAT3 on Ser 727 correlates with the survival of hES cells (Androutsellis-Theotokis et al., 2006). Thus, STAT3 could play a role in monkey ES cell self-renewal, even though they are unresponsive to LIF.

Several growth factors have been described as potent self-renewal inducers for hES cells (FGF2, Nodal/Activin, TGFβ1 and Wnt); however, the combination of FGF and Activin signalling is the most common and effective culture condition for the maintenance of primate ES cells (Lanner and Rossant, 2010). HES cells express receptors for FGFs and produce FGF2, which activates signalling through the receptor tyrosine kinases, ERK1 and ERK2, in these cells (Dvorak et al., 2005). Inhibition of this signalling pathway or removal of FGF2 from the medium induce human and monkey ES cells differentiation (Mitalipov et al., 2006). Conversely, FGF2 allows the clonal growth of human (Amit et al., 2000) and monkey ES cells (Wianny et al., 2008) on feeder cells, and in feeder-free conditions (Xu et al., 2001; Levenstein et al., 2005; Wang et al., 2005; Xu et al., 2005). Activin and Nodal have been shown to suppress the differentiation of hES cells (Vallier et al., 2004, 2005). Nodal and Activin signal through the same receptors (ACVR1B and ACVR2B), which are expressed in hES cells (Assou et al., 2007). This complex activates the transcription factors Smad2/Smad3, and expression of the key pluripotency transcription factor Nanog occurs downstream of this signalling cascade (Vallier et al., 2009). Smad2 and Smad3 bind to the promoter of the gene encoding Nanog and activate its expression (Xu et al., 2008). Activin and Nodal can synergize with FGF2 to promote hES cell self-renewal. Indeed, hES cells cultured in serum free condition supplemented with activin produce FGF2 (Xiao et al., 2006). Activation of SMAD2- and/or SMAD3-mediated signalling or FGF2-mediated signalling suppresses BMP4 expression in hES cells, preventing spontaneous differentiation (Greber et al., 2007). These observations suggest that both Activin and FGF signalling pathways need to be activated for hES cell maintenance.

2.5. Genetic labelling

In order to study the integration of the grafted cells in the host tissue, it is essential to be able to trace ES cell derivatives *in vivo*. Investigators have used vital tracers to label monkey ES cells. For example, they introduced GFP using lipofection, electroporation (Takada et al., 2002; Ueda et al., 2006), or lentiviral infection (Asano et al., 2002). We have genetically labelled Rhesus ES cells using a simian immunodeficiency virus (SIV)-based lentiviral vector expressing GFP or tau-GFP under the control of the cytomegalovirus enhanced chicken β-actin (CAG) promoter. After cell sorting, we have derived pure populations of GFP or tau-GFP positive ES cells that were similar to their wild-type counterparts. This type of stable labelling constitutes a powerful tool for a wide variety of applications, including developmental studies for tracing the fate monkey ES cells in chimeras, as well as the development of many transplantation technologies in monkey (Wianny et al., 2008).

2.6. Pluripotency

Monkey ES cells are considered to be pluripotent because they can produce differentiated derivatives representative of the three germ layers (ectoderm, endoderm and mesoderm). *In vitro*, this can be achieved (i) by culturing ES cells in sub-confluent conditions, (ii) by testing their capacity to form three-dimensional structures (the so-called "embryoid bodies") composed of a various types of differentiated cells, (iii) by adding exogenous factors, or (iv) by using a combination of these three strategies. It is then possible to drive monkey ES cell differentiation into various cell types *in vitro*, like cardiac and hematopoietic cells, retinal cells, pigmented epithelium, pancreatic cells, and various neural cell types (Kawasaki et al., 2002; Kuo et al., 2003; Honig et al., 2004; Lester et al., 2004; Umeda et al., 2004; Mitalipov et al., 2006; Schwanke et al., 2006; Osakada et al., 2008).

However, to test the pluripotent nature of ES cells, it is sometimes most appropriate to examine cell differentiation *in vivo* after cell transplantation, where grafted cells experience a rich combination of signals from the local environment. For these types of studies, ES cells are transplanted in the kidney capsule or in the testis of immunocompetent mice. Teratomas resulting from transplanted monkey ES cells consist of an array of differentiated tissues representative of all three germ layers, recapitulating some aspects of the embryonic organogenesis (Thomson et al., 1995; Suemori et al., 2001; Wianny et al., 2008). Thus, monkey ES cells represent a very useful tool for studying the cell biology of early developmental processes. This is particularly attractive in this species in which research on embryos is impractical. The ultimate test for demonstrating the full developmental competence of ES cells is to evaluate their capacity to contribute to embryonic and adult cell lineages in chimeric animals. In a recent study, monkey ES cells labelled with a fluorescent dye (PKH-26) contributed to some extent to expanded blastocyst stage development after aggregation with 4-cell stage embryos. However, upon reimplantation of the chimeric embryo into a recipient female, no newborn monkeys could be obtained (Mitalipov et al., 2006). PKH-26 is rapidly diluted over cell divisions, all the more in actively proliferating ES cells. ES cells genetically modified to express a reporter gene like GFP will obviously be essential for the long term tracing of ES cell descendants at the pre-implantation stage, and also in the foetuses or offspring.

In the following section, we will comment on the capacity of monkey ES cell to enter into early neural differentiation processes, and to follow the different routes of neuronal diversification.

Please cite this article as: Wianny, F., et al., Embryonic stem cells in non-human primates: An overview of neural differentiation potential. Differentiation (2011), doi:10.1016/j.diff.2011.01.008

We will be inspired by the hES cell model, which brings some invaluable information, and has been so far, transposable to the monkey ES model.

3. Differentiation along neural lineages: the lessons from human ES cells

3.1. Early neural differentiation

The potential of monkey ES cells for neural differentiation was first demonstrated in teratomas that were found to include tissue with a resemblance to the neural tube, recapitulating, at least in part, neural development *in vivo* (Thomson et al., 1995). *In vitro*, neural differentiation of monkey and human ES cells can be easily induced by prolonged culture without replacing the mouse embryonic feeder layer (Reubinoff et al., 2001; Zhang et al., 2001; Shin et al., 2006; Tibbitts et al., 2006; Wianny et al., 2008). ES cells then undergo morphogenetic changes characterised by the radial arrangements of columnar cells forming "neural rosettes" reminiscent of the neuroectoderm (NE) (Zhang et al., 2001; Perrier et al., 2004; Pankratz et al., 2007; Wianny et al., 2008). These rosette-like cells express many of the markers of neuroepithelial cells of the neural plate, such as Pax6 and Sox1 and they are capable of differentiating into various region-specific neuronal and glial cell types in response to appropriate developmental signals (Perrier et al., 2004; Li et al., 2005b).

Two main strategies, derived from the mouse model, have been specifically developed to drive monkey and human ES cell differentiation into the neural lineage. A first protocol mimics the interactions between cells during neural specification *in vivo*. Considering that neural specification occurs through interactions with underlying mesoderm cells during embryonic development, monkey ES cells (Kawasaki et al., 2002; Sanchez-Pernaute et al., 2005; Takagi et al., 2005) and hES cells (Perrier et al., 2004; Park et al., 2005; Vazin et al., 2008) were co-cultured and grown on mouse stromal cells (such as bone marrow stroma PA6 cells or MS5 lines). The so-called stromal cell induced activity (SDIA) produced by these stromal cell lines enriched the population of monkey ES cells differentiating into neural progenitors from 0% to nearly 80% after 14 days (Kawasaki et al., 2002; Takagi et al., 2005). Similar results were obtained with hES cells (Perrier et al., 2004; Park et al., 2005; Vazin et al., 2008). The main disadvantage of this protocol is the presence of unknown stromal factors in the cultures, which could obscure the results when one wants to study the mechanisms involved in neural differentiation.

The second strategy involves an embryoid body (EB) intermediate, which initiates the differentiation process. This three-dimensional system recapitulates the environment of early embryo development. A combination of serum free conditions, FGF2 and mechanical isolation enriched the population of monkey ES cells (Calhoun et al., 2003; Kuo et al., 2003) and human ES cells (Zhang et al., 2001; Li et al., 2005b; Pankratz et al., 2007) differentiating into neural precursors, with a 70–90% efficiency among the total differentiated progenies. These cells show a strong proliferative response to FGF2.

3.2. Regional identity

Although neural cells produced with these different protocols appear to be similar, regarding the morphology and the expression of neural markers, they greatly differ in term of regional identity. Neural precursors generated from the SDIA method are generally fated to mid/hindbrain or more caudal lineages, and this method is often used to produce dopaminergic neurons from human (Perrier et al., 2004) and monkey ES cells (Takagi et al., 2005). By contrast, neural precursors obtained from monkey and human EB intermediates and cultured in serum-free conditions exhibit a wider developmental potential. These cells first show anterior characteristics, and express Pax6 (Pankratz et al., 2007). During progressive neural specification, expression of Pax6 is lost and they express Sox1 and anterior patterning genes such as BF1 and Otx2. Interestingly, the initial anterior nature of these neural precursors can be maintained *in vitro*, even in the absence of FGF2 (Li et al., 2005b; Pankratz et al., 2007). This anterior nature can also be re-specified to a posterior fate after treatment with the caudalizing factor retinoic acid (RA), if it is added in a correct time window, during days 10–21 of culture, when primitive neuroectoderm differentiate into definitive neuroectoderm (Pankratz et al., 2007; Zhang et al., 2010). This neural precursor regional plasticity has enabled efficient differentiation of hES cells into spinal motor neurons and midbrain dopamine neurons (Li et al., 2005b; Yan et al., 2005), and will probably open the way for specifying numerous other neural subtypes.

3.3. Isolating multipotent neural stem cell lines

In the context of cell therapy, it is crucial to have a reproducible, reliable and quality controlled population of Neural Stem/Progenitor cells (NSCs) exhibiting true self-renewal, full patterning and differentiation potential. The first attempts to derive *bona fide* self-renewing NSCs from human and monkey ES cells were not completely successful; the neural precursors were capable to be expanded for extended time in culture either as a monolayer (Gerrard et al., 2005; Li et al., 2005a) or in aggregates termed neurospheres, which contain a heterogeneous mix of cells composed of only a small percentage of truly multipotent stem cells (3–4%) (Reubinoff et al., 2001; Banin et al., 2006; Joannides et al., 2007). However, it was not clear whether these cells retain their self-renewal capacity in long-term cultures (Kuo et al., 2003; Li et al., 2005a; Zhang, 2006; Ko et al., 2007). It is only few years ago that several groups reported the successful derivation of NSCs from mouse and hES cells (Conti et al., 2005; Glaser et al., 2007; Daadi et al., 2008; Elkabetz et al., 2008; Spiliotopoulos et al., 2009). These long term self-renewing NSCs, also named NS cells (Conti et al., 2005), or NSCs$^{FGF2/EGF}$ (Elkabetz et al., 2008), express the neural precursor markers Sox2, Sox1 (in human) and emx2, and the radial glial markers Pax6, Glast, BLBP, vimentin and prominin, suggesting that they are closely related to the radial glial lineage, a transient population of multipotent neural stem cells present in the embryonic brain at the beginning of neurogenesis. NSCs$^{FGF2/EGF}$ can be propagated continuously as adherent monolayer in the presence of FGF2/epidermal growth factor (EGF), a growth factor combination that is known to be effective for the propagation of human–foetal and adult derived neural stem cells (Reynolds and Weiss, 1996; Svendsen et al., 1998; Vescovi et al., 1999; Uchida et al., 2000) (Fig. 1). NSCs$^{FGF2/EGF}$ are characterised by their long-term self-renewal, and their capacity to differentiate into electro-physiologically active neurons, astrocytes and oligodendrocytes across several passages (Conti et al., 2005; Daadi et al., 2008; Spiliotopoulos et al., 2009). Importantly, they do not show any chromosomal abnormalities, they are not tumorigenic *in vivo* (Daadi et al., 2008), and they can survive and differentiate in both foetal and adult brain environments. However, these cells progressively lose their competence to produce diverse neuronal subtypes, showing a limited potential to generate motoneuronal and dopaminergic progeny *in vitro* and *in vivo* (Elkabetz et al., 2008) and they become mainly constrained to adopt a GABAergic fate.

Thus, determining the optimal conditions for long-term self-renewal and full differentiation potential of NSCs remains a challenge in primates. Two very recent interesting studies made

Please cite this article as: Wianny, F., et al., Embryonic stem cells in non-human primates: An overview of neural differentiation potential. Differentiation (2011), doi:10.1016/j.diff.2011.01.008

major breakthroughs in this direction, showing that neural rosettes derived from hES cells contain NSCs that can be propagated *in vitro* (Elkabetz et al., 2008; Koch et al., 2009) (Fig. 1). The two types of early NSCs produced by these two groups express the classical NSC markers like Nestin, Sox2, Sox1 and 3CB2. However, they are not equivalent in term of self-renewal capacity, expression of markers and regional identity: the first type of early NSCs described by Elkabetz and collaborators (also called R-NSCs) were isolated from early rosettes and expanded for only a limited number of passages *in vitro* in the presence of SHH and Notch receptor agonists (Elkabetz et al., 2008). They express a large panel of rosette markers, such as Dach1, apical zonula occludens 1 (ZO1), a tight junction protein which is distributed asymmetrically and apically in neural progenitors, and PLZF, known to bind the polycomb group protein BMI-1, a crucial component in NSC self-renewal (Molofsky et al., 2003). Most importantly, because of their remarkable plasticity, R-NSCs are able to adopt the CNS and the PNS fates (Elkabetz et al., 2008): two populations of R-NSCs could be separated on the basis of forebrain surface embryonic-1 (Forse-1) epitope expression: Forse1+R-NSCs which bear an anterior identity and can be re-specified to a caudal fate, and Forse1-R-NSCs that bear a posterior identity with the capacity to generate neural crest lineages. All these properties are reminiscent of neuroectoderm cells at the neural plate stage *in vivo*. In the presence of EGF and FGF2, R-NSCs show a complete loss of rosette morphology, a decrease in R-NSC markers, and a concomitant increase of later stage neural precursor markers S100β and AQP4 reflecting a conversion into $NSCs^{FGF2/EGF}$.

The second type of early NSCs also isolated from human neural rosettes are called long term self-renewing NSCs (or lt-ESNSCs) (Koch et al., 2009). They are maintained in the presence of FGF2, EGF, and a low concentration of the supplement B27. These cells can be extensively propagated while retaining a broad differentiation potential and a normal karyotype. Lt-ESNSCs exhibit rosette-like pattern even after extensive passaging *in vitro* and share the expression of some (but not all) markers with R-NSCs, such as Forse1, Dach1, ZO-1 and PLZF, but lose expression of other markers also described at the rosette stage such as FAM70A, EVI1, ZNF312, LIX1, or RSPO3. Importantly, they do not express some $NSC^{FGF2/EGF}$ markers, such as PMP2, AQP2, SPARCL1, HOP, or S100β. During *in vitro* expansion, lt-ESNSCs show a switch from anterior to more posterior regional identity, and exhibit a regional transcription factor code characteristic of anterior hindbrain cells, probably due to the well-known posteriorizing effect of FGF2. Lt-ESNSCs show a prominent propensity to generate GABAergic neurons, but they exhibit sufficient plasticity to give rise to ventral midbrain TH-positive neurons or even to motoneurons. However, these cells do not seem to have the capacity to be "re-anteriorized" toward a telencephalic fate. Because lt-ESNSCs partially retain rosette properties, and lack expression of markers characteristic of $NSCs^{FGF2/EGF}$, it has been suggested that lt-ESNSCs may represent an intermediate developmental stage between R-NSCs and $NSC^{FGF2/EGF}$.

Notwithstanding, R-NSCs and lt-ESNSCs show major limitations that would compromise their use in cell replacement therapies: R-NSCs exhibit *in vivo* overgrowth when transplanted into the adult brain, and lt-ESNSCs show a restricted differentiation potential. However, the successful establishment of conditions for stable self-renewal and full differentiation potential of NSCs in primates will greatly benefit from these two models.

3.4. Signalling pathways involved in neural induction and maintenance of NSCs

The mechanisms involved in the specification of neuroectoderm and the maintenance of NSCs are orchestrated by multiple players, among which four signalling pathways play a major role during mammalian neurogenesis: the bone morphogenetic protein (BMP), FGF2, Notch and Wnt/β-catenin pathways (Fig. 1). These mechanisms have been mainly deciphered in hES cells.

In agreement with the "default" model of neural induction, which stated that the embryonic ectoderm becomes neuroectoderm when transforming growth factor-β (TGF-β)/BMP/SMAD signalling is inhibited (reviewed by Wilson and Edlund, 2001), strong inhibition of TGF-β signalling induces a rapid neural conversion of hES cells under adherent culture conditions (Pera et al., 2004; Chambers et al., 2009). Similarly, hES cells that over express inhibitors of the Nodal pathway (a TGFβ-related signal), display increased neural induction (Vallier et al., 2004; Smith et al., 2008). Repression of TGFβ signalling may also be critical for the isolation and the maintenance R-NSCs, since these cells express high levels of Dach1 and Evi, which both act as a repressor of TGFβ signalling (Kurokawa et al., 1998; Wu et al., 2003). For this reason, most neural differentiation protocols require ES cells to be grown in the absence of serum to avoid the effect of BMPs.

However, neural induction of hES cells does not simply rely on a BMP antagonism. Other cues play an active role. RA is well-known to be one of the most potent inducers of neural differentiation in both mouse and hES cells, if used in combination with specific culture conditions such as serum-free environment or low-density culture (Carpenter et al., 2001). RA mainly induces caudally fated neuroepithelial cells, and is therefore used to generate specific neuron subtypes (Li et al., 2005b).

FGF2 represent an additional regulatory influence, which may affect the adoption of neural fates. As stated above, the use of FGF2 enables efficient production of neural progenitors from monkey and human ES cells, but it is actually unclear whether it acts on differentiation, proliferation and/or survival of neural precursor cells. EGF together with FGF2 is essential for the derivation and propagation of NS cells (Conti et al., 2005).

Interestingly, it was found that FGF2 acts in synergy with Notch signalling during early neural specification of mouse ES cells (Lowell et al., 2006). In human, activation of Notch signalling induces differentiation of ES cells into neuroectoderm. Fibroblasts engineered to express Notch ligand Delta 1 promoted neural specification hES cells. Conversely, disruption of Notch signalling reduced commitment of ES cells to the neural lineage (Lowell et al., 2006). Notch signalling is also known to play a central role in the maintenance of neural precursors/NSCs *in vivo* in the mouse (Yoshimatsu et al., 2006; Cao et al. 2010), and in human (Hansen et al. 2010). In line with this property, Notch was found to be crucial for the maintenance of derived-R-NSCs and $NSCs^{FGF2/EGF}$ (Elkabetz et al., 2008; Borghese et al. 2010). Treatment of NSCs with DAPT, an inhibitor of Notch receptors processing and activation, causes premature neuronal differentiation, and slows down progression through G1/S-phase of the cell cycle. Conversely, activation of Notch signalling using the Notch ligands Dll4 and Jag1 enhanced maintenance of R-NSC state and proliferation (Elkabetz et al., 2008). In the mouse and human, STAT3 was also found to be necessary for NSC maintenance, and inhibition of neurogenesis (Chojnacki et al., 2003; Nagao et al., 2007; Cao et al. 2010), and interestingly, activation of JAK-STAT signalling pathway rapidly increased Notch1 expression and activation of Notch signalling (Nagao et al., 2007). Phosphorylation on Ser 727 of STAT3 was proposed to be the mechanisms underlying the crosstalk between Notch and STAT3 signalling (Androutsellis-Theotokis et al., 2006). STAT3 is actually required to maintain the expression of dll1, and elimination of STAT3 reduced ddl1 levels and Notch activation, resulting in the promotion of neurogenesis (Yoshimatsu et al., 2006).

In the monkey, we found that neural precursors and $NSCs^{FGF2/EGF}$ derived from LYON-ES1 cell line (Wianny et al., 2008),

Please cite this article as: Wianny, F., et al., Embryonic stem cells in non-human primates: An overview of neural differentiation potential. Differentiation (2011), doi:10.1016/j.diff.2011.01.008

using the established protocols (Conti et al., 2005; Glaser et al., 2007), express components of Notch and STAT3 signalling pathways, suggesting that these pathways are active during neural induction and could play an active role in the maintenance of monkey NSCs.

Sonic hedgehog (SHH) signalling is known to be important for the maintenance of foetal and adult NSCs *in vivo* and *in vitro* (Rowitch et al., 1999; Machold et al., 2003; Favaro et al., 2009). Similarly, treatment of R-NSCs derived from hES cells with SHH agonists induced robust increase in lumen size and overall rosette growth and the addition of SHH in combination with Notch agonists was found to be optimal for long-term R-NSC maintenance (Elkabetz et al., 2008). A very recent study showed that the effects of SHH signalling on NSC formation depend on the induction of Sox9 in both the embryonic and adult mouse CNS. Gain- and loss-of-function studies showed that SOX9 was essential for the generation and maintenance of multipotent NSCs in the embryo and the generation of neurospheres *in vitro*. Induction of Sox9 in neural progenitors cells using genetic tools converts them into NSCs *in vitro* and *in vivo* (Scott et al.). Similarly, exposure of neural progenitors to SHH induced Sox9 transcription and resulted in cells precociously differentiating into NSCs *in vitro*. The expression of Sox9 is up regulated during early neural differentiation of hES cells (Pankratz et al., 2007), and it is expressed in NSCs derived from monkey ES cells (our unpublished observations). It is then tempting to hypothesise that Sox9 will also play an important role in neural induction and maintenance of NSCs derived from primate ES cells.

Wnt/β Catenin signalling is another essential regulator of neural induction and maintenance of NSCs (extensively reviewed by Michaelidis and Lie, 2008). Interestingly, induction of hES cell neural differentiation was associated with an increase in the expression of Wnt/β Catenin signalling components such as Wnt5B, frizzled 1 and 4, TLE4 and TCF7L2 (Pankratz et al., 2007).

In search of identifying transcriptional determinants underlying mammalian neuroectoderm specification, Zhang and collaborators found that Pax6 was necessary and sufficient for neuroectoderm specification from human but not mouse ES cells (Zhang et al., 2010). Knockdown of Pax6 blocks neuroectoderm specification from hES cells which then retain pluripotent gene expression. This neural inductive function of Pax6 is achieved by its repression of pluripotent genes (role played by the two isoforms Pax6a and Pax6b), and activation of neuroectoderm genes (role played by Pax6a only). Over expression of Pax6a induced neural rosette formation within hES cell colonies, and this is accompanied by up regulation of (i) forebrain associated genes, such as genes playing crucial roles in eye development (Six3, Lhx2), (ii) genes abundantly expressed in foetal brain (BLBP), (iii) genes involved in the Wnt/β-catenin signalling pathway (Wnt5B, Frizzled 1 and 4), and (iv) in the inhibition of BMP/Smad signalling. Pax6 may thus be a crucial downstream effector of neural inducers.

3.5. From neural induction to cortical differentiation

In the past few years, differentiation of ES cell into the different cortical cell types has been used as a model to study the spatiotemporal events occurring during embryonic corticogenesis, not only in the mouse (Watanabe et al., 2005; Eiraku et al., 2008), but also in human (Gaspard et al., 2008). This strategy developed in the human will without any doubt be transposable to the monkey. In the monkey, cortical development starts to be deciphered (Lukaszewicz et al., 2005, 2006; Dehay and Kennedy, 2007), and a better understanding of the signalling pathways that control cortical development in NHP is necessary to achieve proper differentiation of monkey ES cells into cortical neural subtypes. The understanding of these molecular mechanisms using these types of *in vitro* models will provide new insights into the regulation of neural induction and cortical development in the early monkey embryo. In the context of regenerative medicine, it represents an important step forward in the effective production of specific cortical neurons that are lost in various neuronal diseases, like Amyotrophic lateral sclerosis and Alzheimer disease, and for testing their therapeutical properties in NHP.

3.6. Neuronal differentiation of monkey ES cells and their behaviour in vivo

Neural precursors derived from monkey ES cells were found to have the property to differentiate into various neural cell types, including neurons, astrocytes and oligodendrocytes (Calhoun et al., 2003; Kuo et al., 2003; Li et al., 2005a; Takagi et al., 2005; Tibbitts et al., 2006; Wianny et al., 2008). Amongst these neural cell types, some specific cell types could be produced in a relatively high purity, including dopaminergic neurons, motor neurons, striatal interneurons, and oligodendrocytes (Ikeda et al., 2005; Sanchez-Pernaute et al., 2005; Takagi et al., 2005), which are the main degenerating cells in Parkinson's disease (PD), amyotrophic lateral sclerosis, Huntington's disease, and multiple sclerosis, respectively. In the case of PD models, protocols used to obtain dopaminergic cells from monkey ES cells were adapted from the human model (Ben-Hur et al., 2004; Perrier et al., 2004; Zeng et al., 2004; Correia et al., 2005; Park et al., 2005; Yan et al., 2005; Roy et al., 2006; Iacovitti et al., 2007; Yang et al., 2008). Takagi et al. (2005) obtained midbrain dopaminergic (DA) progenitors by treating monkey ES cell derived neural precursors with FGF2 and FGF20 (a secreted protein that enhances the survival of primary DA neurons) (Ohmachi et al., 2000). This treatment greatly improved the percentage of cells expressing Tyrosine hydroxylase (TH), an enzyme expressed in dopaminergic neurons. *In vitro*, 24% of post-mitotic neurons expressed this enzyme. These DA progenitors also strongly express midbrain DA neuron markers such as Pax2, Ptx3, Nurr1 and Lmx1b. They produce enzymes related to dopamine metabolism such as aromatic amino acid decarboxylase (AADC) and vesicular monoamine transporter 2 (VMAT2), and they release dopamine in response to high K+ depolarising stimuli, which altogether are good markers for functionality. Importantly, upon transplantation in a Parkinsonian monkey brain, DA progenitors were found to function as dopaminergic neurons, and to attenuate neurological symptoms (Takagi et al., 2005). Midbrain DA neurons were also obtained using SDIA induction protocol (Kawasaki et al., 2002; Sanchez-Pernaute et al., 2005). In response to FGF8, a mid/hindbrain organising morphogen, and SHH, a ventralizing morphogen, neural precursors differentiate robustly into DA neurons *in vitro*, with a yield of 35–60% cells expressing TH, Nurr1 and Lmx1b and producing significant amounts of dopamine. However, upon grafting into the Parkinsonian rat or monkey brain, survival of these mature DA neurons was poor (0.1%), and no behavioural amelioration was observed (Sanchez-Pernaute et al., 2005). DA progenitors show a higher survival rate (25%) upon grafting in the brain (Li et al., 2005a; Takagi et al., 2005). This is probably due to their higher resistance to mechanical stress, compared with fully matured DA neurons. Other types of neurons are frequently observed in cultures designed for dopamine neuron differentiation. For example, SDIA-treated primate ES cells gave rise, in addition to TH neurons, to GABAergic (20% of post-mitotic neurons), cholinergic (5%), and serotonergic (1%) neurons. Using a relatively simple protocol, Salli and collaborators reported successful differentiation of rhesus ES cells into a highly enriched population of serotonin like neurons, expressing

Please cite this article as: Wianny, F., et al., Embryonic stem cells in non-human primates: An overview of neural differentiation potential. Differentiation (2011), doi:10.1016/j.diff.2011.01.008

ARTICLE IN PRESS

serotonergic markers like the serotonin-synthesising enzyme, the tryptophan hydroxylase (TPH) and the serotonin reuptake transporter (SERT). These cells also secrete serotonin (Salli et al., 2004).

To obtain spinal cord motor neurons in the monkey, neural precursors are "instructed" by RA (Ikeda et al., 2005; Hayashi et al., 2006). 50% of the cells differentiate into islet1+motoneurons. Transplantation of these motoneurons in the brain of hemiplegic experimentally injured mice improved their motor function.

Neural precurcors and NSCs derived from monkey ES cells have the potential to yield all cell types that constitute a normal anatomical brain structure (neurons, astrocytes and "chaperone"-like cells). Thus, in the case of PD models, these cells are not only capable to replace host degenerating DA neurons, but may also provide trophic, protective and guidance effect that promote homoeostatic adjustment of host nigral DA neurons. Such donor–host interactions may result in equilibrium among the DA-related structures, which could benefit behavioural recovery. In the future, the use of postsynaptic markers, such as fluoroclopride, should allow a better understanding of the functional aspects of the grafted cells, in respect with the connections with the surrounding neurons.

Currently, ES cell-based therapies still face several hurdles. The first trials in monkey reveal two major problems: the low survival rate of the grafted cells and the disappointing functional outcome upon grafting into the lesioned brain. Much still needs to be done in the monkey, where few studies have been reported regarding the therapeutic potential of ES cell derivatives. Furthermore, in these studies, donor cells derived from monkey ES cells were implanted for practical reasons directly into the striatum, the site of lost dopaminergic input. However, the implanted cells did not substantially migrate beyond the striatum in the rat and in the monkey (Takagi et al., 2005; Sanchez-Pernaute et al., 2005). Similar observations were made when hES cell-derived neural precursors were implanted in the rat brain (Ben-Hur et al., 2004; Brederlau et al., 2006). An alternative strategy would be to transplant cells at the site from which the cell bodies are lost, into the substantia nigra (SN), or use multitarget graft strategy, where cells would be transplanted into numerous sites along the nigrostriatal pathway. This type of strategy recently used to study the migration of human foetal NSCs upon grafting into the monkey brain gave encouraging results (Bjugstad et al., 2008), and may offer an important adjunct to other cell-based strategies.

4. Concluding remarks

NHP ES cells and hES cells show extensive similarities, and most of the studies done so far using hES cells are transposable to monkey ES cells. Mouse ES cells do not share these properties. For this reason, NHP ES cells provide more pertinent information than mouse ES cells, especially in the case of transplantation studies. While the transplantation of hES cell derivatives into patients is limited by ethical and safety concerns (reviewed in Hentze et al., 2007; Li et al., 2008), NHP ES cells can be used to address crucial issues before any clinical trials, and for this reason they provide an invaluable translational model. These issues mainly concern the survival and the integration of the grafted cells in the host, the stability of their phenotype after grafting, as well as their tumorigenicity.

Another important feature of NHP ES cells is their differentiation potential into specific developmental programs *in vitro*. For this reason, they represent a versatile tool for investigating early developmental processes, such as early corticogenesis, which are less accessible *in vivo* in the primate. Indeed, monkey brain development show features that are very specific to primates (Dehay and Kennedy, 2007), and cannot be studied in the mouse. Such *in vitro* models may bring precious information regarding the identification and the regulation of the molecular events that take place during the specification of cortical areas in the primate brain (Monuki and Walsh, 2001; Hamasaki et al., 2004; Dehay and Kennedy, 2007, 2009).

Acknowledgements

We thank all the members of Colette Dehay and Pierre Savatier teams, at the SBRI for their support.

References

Amit, M., Carpenter, M.K., Inokuma, M.S., Chiu, C.P., Harris, C.P., Waknitz, M.A., Itskovitz-Eldor, J., Thomson, J.A., 2000. Clonally derived human embryonic stem cell lines maintain pluripotency and proliferative potential for prolonged periods of culture. Dev. Biol. 227, 271–278.

Androutsellis-Theotokis, A., Leker, R.R., Soldner, F., Hoeppner, D.J., Ravin, R., Poser, S.W., Rueger, M.A., Bae, S.K., Kittappa, R., McKay, R.D., 2006. Notch signalling regulates stem cell numbers in vitro and in vivo. Nature 442, 823–826.

Asano, T., Hanazono, Y., Ueda, Y., Muramatsu, S., Kume, A., Suemori, H., Suzuki, Y., Kondo, Y., Harii, K., Hasegawa, M., Nakatsuji, N., Ozawa, K., 2002. Highly efficient gene transfer into primate embryonic stem cells with a simian lentivirus vector. Mol. Ther. 6, 162–168.

Assou, S., Le Carrour, T., Tondeur, S., Strom, S., Gabelle, A., Marty, S., Nadal, L., Pantesco, V., Reme, T., Hugnot, J.P., Gasca, S., Hovatta, O., Hamamah, S., Klein, B., De Vos, J., 2007. A meta-analysis of human embryonic stem cells transcriptome integrated into a web-based expression atlas. Stem Cells 25, 961–973.

Banin, E., Obolensky, A., Idelson, M., Hemo, I., Reinhardtz, E., Pikarsky, E., Ben-Hur, T., Reubinoff, B., 2006. Retinal incorporation and differentiation of neural precursors derived from human embryonic stem cells. Stem Cells 24, 246–257.

Becker, K.A., Ghule, P.N., Therrien, J.A., Lian, J.B., Stein, J.L., van Wijnen, A.J., Stein, G.S., 2006. Self-renewal of human embryonic stem cells is supported by a shortened G1 cell cycle phase. J. Cell Physiol. 209, 883–893.

Becker, K.A., Stein, J.L., Lian, J.B., van Wijnen, A.J., Stein, G.S., 2010. Human embryonic stem cells are pre-mitotically committed to self-renewal and acquire a lengthened G1 phase upon lineage programming. J. Cell Physiol. 222, 103–110.

Ben-Hur, T., Idelson, M., Khaner, H., Pera, M., Reinhartz, E., Itzik, A., Reubinoff, B.E., 2004. Transplantation of human embryonic stem cell-derived neural progenitors improves behavioral deficit in Parkinsonian rats. Stem Cells 22, 1246–1255.

Bjugstad, K.B., Teng, Y.D., Redmond Jr., D.E., Elsworth, J.D., Roth, R.H., Cornelius, S.K., Snyder, E.Y., Sladek Jr., J.R., 2008. Human neural stem cells migrate along the nigrostriatal pathway in a primate model of Parkinson's disease. Exp. Neurol. 211, 362–369.

Boeuf, H., Hauss, C., Graeve, F.D., Baran, N., Kedinger, C., 1997. Leukemia inhibitory factor-dependent transcriptional activation in embryonic stem cells. J. Cell Biol. 138, 1207–1217.

Borghese, L., Dolezalova, D., Opitz, T., Haupt, S., Leinhaas, A., Steinfarz, B., Koch, P., Edenhofer, F., Hampl, A., Brustle, O., 2010. Inhibition of notch signaling in human embryonic stem cell-derived neural stem cells delays G1/S phase transition and accelerates neuronal differentiation in vitro and in vivo. Stem Cells 28, 955–964.

Brederlau, A., Correia, A.S., Anisimov, S.V., Elmi, M., Paul, G., Roybon, L., Morizane, A., Bergquist, F., Riebe, I., Nannmark, U., Carta, M., Hanse, E., Takahashi, J., Sasai, Y., Funa, K., Brundin, P., Eriksson, P.S., Li, J.Y., 2006. Transplantation of human embryonic stem cell-derived cells to a rat model of Parkinson's disease: effect of in vitro differentiation on graft survival and teratoma formation. Stem Cells 24, 1433–1440.

Burdon, T., Smith, A., Savatier, P., 2002. Signalling, cell cycle and pluripotency in embryonic stem cells. Trends Cell Biol. 12, 432–438.

Byrne, J.A., Mitalipov, S.M., Clepper, L., Wolf, D.P., 2006. Transcriptional profiling of rhesus monkey embryonic stem cells. Biol. Reprod. 75, 908–915.

Calhoun, J.D., Lambert, N.A., Mitalipova, M.M., Noggle, S.A., Lyons, I., Condie, B.G., Stice, S.L., 2003. Differentiation of rhesus embryonic stem cells to neural progenitors and neurons. Biochem. Biophys. Res. Commun. 306, 191–197.

Cao, F., Hata, R., Zhu, P., Nakashiro, K., Sakanaka, M., 2010. Conditional deletion of Stat3 promotes neurogenesis and inhibits astrogliogenesis in neural stem cells. Biochem. Biophys. Res. Commun. 394, 843–847.

Carpenter, M.K., Inokuma, M.S., Denham, J., Mujtaba, T., Chiu, C.P., Rao, M.S., 2001. Enrichment of neurons and neural precursors from human embryonic stem cells. Exp. Neurol. 172, 383–397.

Chambers, S.M., Fasano, C.A., Papapetrou, E.P., Tomishima, M., Sadelain, M., Studer, L., 2009. Highly efficient neural conversion of human ES and iPS cells by dual inhibition of SMAD signaling. Nat. Biotechnol. 27, 275–280.

Chojnacki, A., Shimazaki, T., Gregg, C., Weinmaster, G., Weiss, S., 2003. Glycoprotein 130 signaling regulates Notch1 expression and activation in the self-renewal of mammalian forebrain neural stem cells. J. Neurosci. 23, 1730–1741.

Please cite this article as: Wianny, F., et al., Embryonic stem cells in non-human primates: An overview of neural differentiation potential. Differentiation (2011), doi:10.1016/j.diff.2011.01.008

Cibelli, J.B., Grant, K.A., Chapman, K.B., Cunniff, K., Worst, T., Green, H.L., Walker, S.J., Gutin, P.H., Vilner, L., Tabar, V., Dominko, T., Kane, J., Wettstein, P.J., Lanza, R.P., Studer, L., Vrana, K.E., West, M.D., 2002. Parthenogenetic stem cells in nonhuman primates. Science 295, 819.

Conti, L., Pollard, S.M., Gorba, T., Reitano, E., Toselli, M., Biella, G., Sun, Y., Sanzone, S., Ying, Q.L., Cattaneo, E., Smith, A., 2005. Niche-independent symmetrical self-renewal of a mammalian tissue stem cell. PLoS Biol. 3, e283.

Correia, A.S., Anisimov, S.V., Li, J.Y., Brundin, P., 2005. Stem cell-based therapy for Parkinson's disease. Ann. Med. 37, 487–498.

Daadi, M.M., Maag, A.L., Steinberg, G.K., 2008. Adherent self-renewable human embryonic stem cell-derived neural stem cell line: functional engraftment in experimental stroke model. PLoS One 3, e1644.

Daheron, L., Opitz, S.L., Zaehres, H., Lensch, M.W., Andrews, P.W., Itskovitz-Eldor, J., Daley, G.Q., 2004. LIF/STAT3 signaling fails to maintain self-renewal of human embryonic stem cells. Stem Cells 22, 770–778.

Dehay, C., Kennedy, H., 2007. Cell-cycle control and cortical development. Nat. Rev. Neurosci. 8, 438–450.

Dehay, C., Kennedy, H., 2009. Transcriptional regulation and alternative splicing make for better brains. Neuron 62, 455–457.

Draper, J.S., Smith, K., Gokhale, P., Moore, H.D., Maltby, E., Johnson, J., Meisner, L., Zwaka, T.P., Thomson, J.A., Andrews, P.W., 2004. Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. Nat. Biotechnol. 22, 53–54.

Dvorak, P., Dvorakova, D., Koskova, S., Vodinska, M., Najvirtova, M., Krekac, D., Hampl, A., 2005. Expression and potential role of fibroblast growth factor 2 and its receptors in human embryonic stem cells. Stem Cells 23, 1200–1211.

Eiraku, M., Watanabe, K., Matsuo-Takasaki, M., Kawada, M., Yonemura, S., Matsumura, M., Wataya, T., Nishiyama, A., Muguruma, K., Sasai, Y., 2008. Self-organized formation of polarized cortical tissues from ESCs and its active manipulation by extrinsic signals. Cell Stem Cell 3, 519–532.

Elkabetz, Y., Panagiotakos, G., Al Shamy, G., Socci, N.D., Tabar, V., Studer, L., 2008. Human ES cell-derived neural rosettes reveal a functionally distinct early neural stem cell stage. Genes Dev. 22, 152–165.

Evans, M.J., Kaufman, M.H., 1981. Establishment in culture of pluripotential cells from mouse embryos. Nature 292, 154–156.

Favaro, R., Valotta, M., Ferri, A.L., Latorre, E., Mariani, J., Giachino, C., Lancini, C., Tosetti, V., Ottolenghi, S., Taylor, V., Nicolis, S.K., 2009. Hippocampal development and neural stem cell maintenance require Sox2-dependent regulation of Shh. Nat. Neurosci. 12, 1248–1256.

Filipczyk, A.A., Laslett, A.L., Mummery, C., Pera, M.F., 2007. Differentiation is coupled to changes in the cell cycle regulatory apparatus of human embryonic stem cells. Stem Cell Res. 1, 45–60.

Fluckiger, A.C., Marcy, G., Marchand, M., Negre, D., Cosset, F.L., Mitalipov, S., Wolf, D., Savatier, P., Dehay, C., 2006. Cell cycle features of primate embryonic stem cells. Stem Cells 24, 547–556.

Gaspard, N., Bouschet, T., Hourez, R., Dimidschstein, J., Naeije, G., van den Ameele, J., Espuny-Camacho, I., Herpoel, A., Passante, L., Schiffmann, S.N., Gaillard, A., Vanderhaeghen, P., 2008. An intrinsic mechanism of corticogenesis from embryonic stem cells. Nature 455, 351–357.

Gerrard, L., Rodgers, L., Cui, W., 2005. Differentiation of human embryonic stem cells to neural lineages in adherent culture by blocking bone morphogenetic protein signaling. Stem Cells 23, 1234–1241.

Glaser, T., Pollard, S.M., Smith, A., Brustle, O., 2007. Tripotential differentiation of adherently expandable neural stem (NS) cells. PLoS One 2, e298.

Greber, B., Lehrach, H., Adjaye, J., 2007. Fibroblast growth factor 2 modulates transforming growth factor beta signaling in mouse embryonic fibroblasts and human ESCs (hESCs) to support hESC self-renewal. Stem Cells 25, 455–464.

Hamasaki, T., Leingartner, A., Ringstedt, T., O'Leary, D.D., 2004. EMX2 regulates sizes and positioning of the primary sensory and motor areas in neocortex by direct specification of cortical progenitors. Neuron 43, 359–372.

Hansen, D.V., Lui, J.H., Parker, P.R., Kriegstein, A.R., 2010. Neurogenic radial glia in the outer subventricular zone of human neocortex. Nature 464, 554–561.

Hayashi, J., Takagi, Y., Fukuda, H., Imazato, T., Nishimura, M., Fujimoto, M., Takahashi, J., Hashimoto, N., Nozaki, K., 2006. Primate embryonic stem cell-derived neuronal progenitors transplanted into ischemic brain. J. Cereb. Blood Flow Metab. 26, 906–914.

Hentze, H., Graichen, R., Colman, A., 2007. Cell therapy and the safety of embryonic stem cell-derived grafts. Trends Biotechnol. 25, 24–32.

Hogan, B., Tilly, R., 1977. In vitro culture and differentiation on normal mouse blastocysts. Nature 265, 626–629.

Honig, G.R., Li, F., Lu, S.J., Vida, L., 2004. Hematopoietic differentiation of rhesus monkey embryonic stem cells. Blood Cells Mol. Dis. 32, 5–10.

Humphrey, R.K., Beattie, G.M., Lopez, A.D., Bucay, N., King, C.C., Firpo, M.T., Rose-John, S., Hayek, A., 2004. Maintenance of pluripotency in human embryonic stem cells is STAT3 independent. Stem Cells 22, 522–530.

Iacovitti, L., Donaldson, A.E., Marshall, C.E., Suon, S., Yang, M., 2007. A protocol for the differentiation of human embryonic stem cells into dopaminergic neurons using only chemically defined human additives: studies in vitro and in vivo. Brain Res. 1127, 19–25.

Ikeda, R., Kurokawa, M.S., Chiba, S., Yoshikawa, H., Ide, M., Tadokoro, M., Nito, S., Nakatsuji, N., Kondoh, Y., Nagata, K., Hashimoto, T., Suzuki, N., 2005. Transplantation of neural cells derived from retinoic acid-treated cynomolgus monkey embryonic stem cells successfully improved motor function of hemiplegic mice with experimental brain injury. Neurobiol. Dis. 20, 38–48.

Imreh, M.P., Gertow, K., Cedervall, J., Unger, C., Holmberg, K., Szoke, K., Csoregh, L., Fried, G., Dilber, S., Blennow, E., Ahrlund-Richter, L., 2006. In vitro culture conditions favoring selection of chromosomal abnormalities in human ES cells. J. Cell Biochem. 99, 508–516.

Joannides, A.J., Fiore-Heriche, C., Battersby, A.A., Athauda-Arachchi, P., Bouhon, I.A., Williams, L., Westmore, K., Kemp, P.J., Compston, A., Allen, N.D., Chandran, S., 2007. A scaleable and defined system for generating neural stem cells from human embryonic stem cells. Stem Cells 25, 731–737.

Kawasaki, H., Suemori, H., Mizuseki, K., Watanabe, K., Urano, F., Ichinose, H., Haruta, M., Takahashi, M., Yoshikawa, K., Nishikawa, S.I., Nakatsuji, N., Sasai, Y., 2002. Generation of dopaminergic neurons and pigmented epithelia from primate ES cells by stromal cell-derived inducing activity. Proc. Natl. Acad. Sci. USA 29, 29.

Ko, J.Y., Park, C.H., Koh, H.C., Cho, Y.H., Kyhm, J.H., Kim, Y.S., Lee, I., Lee, Y.S., Lee, S.H., 2007. Human embryonic stem cell-derived neural precursors as a continuous, stable, and on-demand source for human dopamine neurons. J. Neurochem. 103, 1417–1429.

Koch, P., Opitz, T., Steinbeck, J.A., Ladewig, J., Brustle, O., 2009. A rosette-type, self-renewing human ES cell-derived neural stem cell with potential for in vitro instruction and synaptic integration. Proc. Natl. Acad. Sci. USA 106, 3225–3230.

Kuo, H.C., Pau, K.Y., Yeoman, R.R., Mitalipov, S.M., Okano, H., Wolf, D.P., 2003. Differentiation of monkey embryonic stem cells into neural lineages. Biol. Reprod. 68, 1727–1735.

Kurokawa, M., Mitani, K., Irie, K., Matsuyama, T., Takahashi, T., Chiba, S., Yazaki, Y., Matsumoto, K., Hirai, H., 1998. The oncoprotein Evi-1 represses TGF-beta signalling by inhibiting Smad3. Nature 394, 92–96.

Lanner, F., Rossant, J., 2010. The role of FGF/Erk signaling in pluripotent cells. Development 137, 3351–3360.

Lerou, P.H., Daley, G.Q., 2005. Therapeutic potential of embryonic stem cells. Blood Rev. 19, 321–331.

Lester, L.B., Kuo, H.C., Andrews, L., Nauert, B., Wolf, D.P., 2004. Directed differentiation of rhesus monkey ES cells into pancreatic cell phenotypes. Reprod. Biol. Endocrinol. 2, 42.

Levenstein, M.E., Ludwig, T.E., Xu, R.H., Llanas, R.A., Vandenheuvel-Kramer, K., Manning, D., and Thomson, J.A. (2005) Basic FGF support of human embryonic stem cell self-renewal. Stem Cells.

Li, J.Y., Christophersen, N.S., Hall, V., Soulet, D., Brundin, P., 2008. Critical issues of clinical human embryonic stem cell therapy for brain repair. Trends Neurosci. 31, 146–153.

Li, T., Zheng, J., Xie, Y., Wang, S., Zhang, X., Li, J., Jin, L., Ma, Y., Wolf, D.P., Zhou, Q., Ji, W., 2005a. Transplantable neural progenitor populations derived from rhesus monkey embryonic stem cells. Stem Cells 23, 1295–1303.

Li, X.J., Du, Z.W., Zarnowska, E.D., Pankratz, M., Hansen, L.O., Pearce, R.A., Zhang, S.C., 2005b. Specification of motoneurons from human embryonic stem cells. Nat. Biotechnol. 23, 215–221.

Lowell, S., Benchoua, A., Heavey, B., Smith, A.G., 2006. Notch promotes neural lineage entry by pluripotent embryonic stem cells. PLoS Biol. 4, e121.

Lukaszewicz, A., Cortay, V., Giroud, P., Berland, M., Smart, I., Kennedy, H., Dehay, C., 2006. The concerted modulation of proliferation and migration contributes to the specification of the cytoarchitecture and dimensions of cortical areas. Cereb. Cortex 16 (Suppl. 1), i26–34.

Lukaszewicz, A., Savatier, P., Cortay, V., Giroud, P., Huissoud, C., Berland, M., Kennedy, H., Dehay, C., 2005. G1 phase regulation, area-specific cell cycle control, and cytoarchitectonics in the primate cortex. Neuron 47, 353–364.

Machold, R., Hayashi, S., Rutlin, M., Muzumdar, M.D., Nery, S., Corbin, J.G., Gritli-Linde, A., Dellovade, T., Porter, J.A., Rubin, L.L., Dudek, H., McMahon, A.P., Fishell, G., 2003. Sonic hedgehog is required for progenitor cell maintenance in telencephalic stem cell niches. Neuron 39, 937–950.

Maitra, A., Arking, D.E., Shivapurkar, N., Ikeda, M., Stastny, V., Kassauei, K., Sui, G., Cutler, D.J., Liu, Y., Brimble, S.N., Noaksson, K., Hyllner, J., Schulz, T.C., Zeng, X., Freed, W.J., Crook, J., Abraham, S., Colman, A., Sartipy, P., Matsui, S., Carpenter, M., Gazdar, A.F., Rao, M., Chakravarti, A., 2005. Genomic alterations in cultured human embryonic stem cells. Nat. Genet. 37, 1099–1103.

Martin, G.R., 1981. Isolation of a pluripotent cell line from early mouse embryos cultured in medium conditioned by teratocarcinoma stem cells. Proc. Natl. Acad. Sci. USA 78, 7634–7638.

Martin, G.R., Evans, M.J., 1975. Differentiation of clonal lines of teratocarcinoma cells: formation of embryoid bodies in vitro. Proc. Natl. Acad. Sci. USA 72, 1441–1445.

Matsuda, T., Nakamura, T., Nakao, K., Arai, T., Katsuki, M., Heike, T., Yokota, T., 1999. STAT3 activation is sufficient to maintain an undifferentiated state of mouse embryonic stem cells. EMBO J. 18, 4261–4269.

Michaelidis, T.M., Lie, D.C., 2008. Wnt signaling and neural stem cells: caught in the Wnt web. Cell Tissue Res. 331, 193–210.

Mitalipov, S., Kuo, H.C., Byrne, J., Clepper, L., Meisner, L., Johnson, J., Zeier, R., Wolf, D., 2006. Isolation and characterization of novel rhesus monkey embryonic stem cell lines. Stem Cells 24, 2177–2186.

Mitalipov, S.M., 2006. Genomic imprinting in primate embryos and embryonic stem cells. Reprod. Fertil. Dev. 18, 817–821.

Molofsky, A.V., Pardal, R., Iwashita, T., Park, I.K., Clarke, M.F., Morrison, S.J., 2003. Bmi-1 dependence distinguishes neural stem cell self-renewal from progenitor proliferation. Nature 425, 962–967.

Monuki, E.S., Walsh, C.A., 2001. Mechanisms of cerebral cortical patterning in mice and humans. Nat. Neurosci. Suppl. 1199–1206, 4.

Muller, T., Fleischmann, G., Eildermann, K., Matz-Rensing, K., Horn, P.A., Sasaki, E., Behr, R., 2009. A novel embryonic stem cell line derived from the common

Please cite this article as: Wianny, F., et al., Embryonic stem cells in non-human primates: An overview of neural differentiation potential. Differentiation (2011), doi:10.1016/j.diff.2011.01.008

ARTICLE IN PRESS

marmoset monkey (*Callithrix jacchus*) exhibiting germ cell-like characteristics. Hum. Reprod. 24, 1359–1372.

Nagao, M., Sugimori, M., Nakafuku, M., 2007. Cross talk between notch and growth factor/cytokine signaling pathways in neural stem cells. Mol. Cell Biol. 27, 3982–3994.

Navara, C.S., Mich-Basso, J.D., Redinger, C.J., Ben-Yehudah, A., Jacoby, E., Kovkarova-Naumovski, E., Sukhwani, M., Orwig, K., Kaminski, N., Castro, C.A., Simerly, C.R., Schatten, G., 2007. Pedigreed primate embryonic stem cells express homogeneous familial gene profiles. Stem Cells 25, 2695–2704.

Nichols, J., Gardner, R.L., 1984. Heterogeneous differentiation of external cells in individual isolated early mouse inner cell masses in culture. J. Embryol. Exp. Morphol. 80, 225–240.

Niwa, H., Burdon, T., Chambers, I., Smith, A., 1998. Self-renewal of pluripotent embryonic stem cells is mediated via activation of STAT3. Genes Dev. 12, 2048–2060.

Ohmachi, S., Watanabe, Y., Mikami, T., Kusu, N., Ibi, T., Akaike, A., Itoh, N., 2000. FGF-20, a novel neurotrophic factor, preferentially expressed in the substantia nigra pars compacta of rat brain. Biochem. Biophys. Res. Commun. 277, 355–360.

Osakada, F., Ikeda, H., Mandai, M., Wataya, T., Watanabe, K., Yoshimura, N., Akaike, A., Sasai, Y., Takahashi, M., 2008. Toward the generation of rod and cone photoreceptors from mouse, monkey and human embryonic stem cells. Nat. Biotechnol. 26, 215–224.

Pankratz, M.T., Li, X.J., Lavaute, T.M., Lyons, E.A., Chen, X., Zhang, S.C., 2007. Directed neural differentiation of human embryonic stem cells via an obligated primitive anterior stage. Stem Cells 25, 1511–1520.

Papaioannou, V.E., McBurney, M.W., Gardner, R.L., Evans, M.J., 1975. Fate of teratocarcinoma cells injected into early mouse embryos. Nature 258, 70–73.

Park, C.H., Minn, Y.K., Lee, J.Y., Choi, D.H., Chang, M.Y., Shim, J.W., Ko, J.Y., Koh, H.C., Kang, M.J., Kang, J.S., Rhie, D.J., Lee, Y.S., Son, H., Moon, S.Y., Kim, K.S., Lee, S.H., 2005. In vitro and in vivo analyses of human embryonic stem cell-derived dopamine neurons. J. Neurochem. 92, 1265–1276.

Pau, K.Y., Wolf, D.P., 2004. Derivation and characterization of monkey embryonic stem cells. Reprod. Biol. Endocrinol. 2, 41.

Pera, M.F., Andrade, J., Houssami, S., Reubinoff, B., Trounson, A., Stanley, E.G., Ward-van Oostwaard, D., Mummery, C., 2004. Regulation of human embryonic stem cell differentiation by BMP-2 and its antagonist noggin. J. Cell Sci. 117, 1269–1280.

Pera, M.F., Trounson, A.O., 2004. Human embryonic stem cells: prospects for development. Development 131, 5515–5525.

Perrier, A.L., Tabar, V., Barberi, T., Rubio, M.E., Bruses, J., Topf, N., Harrison, N.L., Studer, L., 2004. Derivation of midbrain dopamine neurons from human embryonic stem cells. Proc. Natl. Acad. Sci. USA 101, 12543–12548.

Reubinoff, B.E., Itsykson, P., Turetsky, T., Pera, M.F., Reinhartz, E., Itzik, A., Ben-Hur, T., 2001. Neural progenitors from human embryonic stem cells. Nat. Biotechnol. 19, 1134–1140.

Reynolds, B.A., Weiss, S., 1996. Clonal and population analyses demonstrate that an EGF-responsive mammalian embryonic CNS precursor is a stem cell. Dev. Biol. 175, 1–13.

Rowitch, D.H., B, S.J., Lee, S.M., Flax, J.D., Snyder, E.Y., McMahon, A.P., 1999. Sonic hedgehog regulates proliferation and inhibits differentiation of CNS precursor cells. J. Neurosci. 19, 8954–8965.

Roy, N.S., Cleren, C., Singh, S.K., Yang, L., Beal, M.F., Goldman, S.A., 2006. Functional engraftment of human ES cell-derived dopaminergic neurons enriched by coculture with telomerase-immortalized midbrain astrocytes. Nat. Med. 12, 1259–1268.

Salli, U., Reddy, A.P., Salli, N., Lu, N.Z., Kuo, H.C., Pau, F.K., Wolf, D.P., Bethea, C.L., 2004. Serotonin neurons derived from rhesus monkey embryonic stem cells: similarities to CNS serotonin neurons. Exp. Neurol. 188, 351–364.

Sanchez-Pernaute, R., Studer, L., Ferrari, D., Perrier, A., Lee, H., Vinuela, A., Isacson, O., 2005. Long-term survival of dopamine neurons derived from parthenogenetic primate embryonic stem cells (cyno-1) after transplantation. Stem Cells 23, 914–922.

Sasaki, E., Hanazawa, K., Kurita, R., Akatsuka, A., Yoshizaki, T., Ishii, H., Tanioka, Y., Ohnishi, Y., Suemizu, H., Sugawara, A., Tamaoki, N., Izawa, K., Nakazaki, Y., Hamada, H., Suemori, H., Asano, S., Nakatsuji, N., Okano, H., Tani, K., 2005. Establishment of novel embryonic stem cell lines derived from the common marmoset (*Callithrix jacchus*). Stem Cells 23, 1304–1313.

Savatier, P., Lapillonne, H., Jirmanova, L., Vitelli, L., Samarut, J., 2002. Analysis of the cell cycle in mouse embryonic stem cells. Methods Mol. Biol. 185, 27–33.

Schwanke, K., Wunderlich, S., Reppel, M., Winkler, M.E., Matzkies, M., Groos, S., Itskovitz-Eldor, J., Simon, A.R., Hescheler, J., Haverich, A., Martin, U., 2006. Generation and characterization of functional cardiomyocytes from rhesus monkey embryonic stem cells. Stem Cells 24, 1423–1432.

Scott, C.E., Wynn, S.L., Sesay, A., Cruz, C., Cheung, M., Gomez Gaviro, M.V., Booth, S., Gao, B., Cheah, K.S., Lovell-Badge, R., Briscoe, J., 2010. SOX9 induces and maintains neural stem cells. Nat. Neurosci. 13, 1181–1189.

Shin, S., Mitalipova, M., Noggle, S., Tibbitts, D., Venable, A., Rao, R., Stice, S.L., 2006. Long-term proliferation of human embryonic stem cell-derived neuroepithelial cells using defined adherent culture conditions. Stem Cells 24, 125–138.

Simerly, C.R., Navara, C.S., Castro, C.A., Turpin, J.C., Redinger, C.J., Mich-Basso, J.D., Jacoby, E.S., Grund, K.J., McFarland, D.A., Oliver, S.L., Ben-Yehudah, A., Carlisle, D.L., Frost, P., Penedo, C., Hewitson, L., Schatten, G., 2009. Establishment and characterization of baboon embryonic stem cell lines: an Old World Primate model for regeneration and transplantation research. Stem Cell Res.

Smith, J.R., Vallier, L., Lupo, G., Alexander, M., Harris, W.A., Pedersen, R.A., 2008. Inhibition of Activin/Nodal signaling promotes specification of human embryonic stem cells into neuroectoderm. Dev. Biol. 313, 107–117.

Solter, D., Skreb, N., Damjanov, I., 1970. Extrauterine growth of mouse egg-cylinders results in malignant teratoma. Nature 227, 503–504.

Spiliotopoulos, D., Goffredo, D., Conti, L., Di Febo, F., Biella, G., Toselli, M., Cattaneo, E., 2009. An optimized experimental strategy for efficient conversion of embryonic stem (ES)-derived mouse neural stem (NS) cells into a nearly homogeneous mature neuronal population. Neurobiol. Dis. 34, 320–331.

Stevens, L.C., 1970. The development of transplantable teratocarcinomas from intratesticular grafts of pre- and postimplantation mouse embryos. Dev. Biol. 21, 364–382.

Suemori, H., Tada, T., Torii, R., Hosoi, Y., Kobayashi, K., Imahie, H., Kondo, Y., Iritani, A., Nakatsuji, N., 2001. Establishment of embryonic stem cell lines from cynomolgus monkey blastocysts produced by IVF or ICSI. Dev. Dyn. 222, 273–279.

Sumi, T., Fujimoto, Y., Nakatsuji, N., Suemori, H., 2004. STAT3 is dispensable for maintenance of self-renewal in nonhuman primate embryonic stem cells. Stem Cells 22, 861–872.

Svendsen, C.N., ter Borg, M.G., Armstrong, R.J., Rosser, A.E., Chandran, S., Ostenfeld, T., Caldwell, M.A., 1998. A new method for the rapid and long term growth of human neural precursor cells. J. Neurosci. Methods 85, 141–152.

Takada, T., Suzuki, Y., Kondo, Y., Kadota, N., Kobayashi, K., Nito, S., Kimura, H., Torii, R., 2002. Monkey embryonic stem cell lines expressing green fluorescent protein. Cell Transplant 11, 631–635.

Takagi, Y., Takahashi, J., Saiki, H., Morizane, A., Hayashi, T., Kishi, Y., Fukuda, H., Okamoto, Y., Koyanagi, M., Ideguchi, M., Hayashi, H., Imazato, T., Kawasaki, H., Suemori, H., Omachi, S., Iida, H., Itoh, N., Nakatsuji, N., Sasai, Y., Hashimoto, N., 2005. Dopaminergic neurons generated from monkey embryonic stem cells function in a Parkinson primate model. J. Clin. Invest. 115, 102–109.

Thomson, J.A., Kalishman, J., Golos, T.G., Durning, M., Harris, C.P., Becker, R.A., Hearn, J.P., 1995. Isolation of a primate embryonic stem cell line. Proc. Natl. Acad. Sci. USA 92, 7844–7848.

Thomson, J.A., Kalishman, J., Golos, T.G., Durning, M., Harris, C.P., Hearn, J.P., 1996. Pluripotent cell lines derived from common marmoset (*Callithrix jacchus*) blastocysts. Biol. Reprod. 55, 254–259.

Tibbitts, D., Rao, R.R., Shin, S., West, F.D., Stice, S.L., 2006. Uniform adherent neural progenitor populations from rhesus embryonic stem cells. Stem Cells Dev 15, 200–208.

Uchida, N., Buck, D.W., He, D., Reitsma, M.J., Masek, M., Phan, T.V., Tsukamoto, A.S., Gage, F.H., Weissman, I.L., 2000. Direct isolation of human central nervous system stem cells. Proc. Natl. Acad. Sci. USA 97, 14720–14725.

Ueda, S., Yoshikawa, M., Ouji, Y., Saito, K., Moriya, K., Nishiofuku, M., Hayashi, N., Ishizaka, S., Shimada, K., Konishi, N., Fukui, H., 2006. Cynomolgus monkey embryonic stem cell lines express green fluorescent protein. J. Biosci. Bioeng. 102, 14–20.

Umeda, K., Heike, T., Yoshimoto, M., Shiota, M., Suemori, H., Luo, H.Y., Chui, D.H., Torii, R., Shibuya, M., Nakatsuji, N., Nakahata, T., 2004. Development of primitive and definitive hematopoiesis from nonhuman primate embryonic stem cells in vitro. Development 131, 1869–1879.

Vallier, L., Alexander, M., Pedersen, R.A., 2005. Activin/Nodal and FGF pathways cooperate to maintain pluripotency of human embryonic stem cells. J. Cell Sci. 118, 4495–4509.

Vallier, L., Mendjan, S., Brown, S., Chng, Z., Teo, A., Smithers, L.E., Trotter, M.W., Cho, C.H., Martinez, A., Rugg-Gunn, P., Brons, G., Pedersen, R.A., 2009. Activin/Nodal signalling maintains pluripotency by controlling Nanog expression. Development 136, 1339–1349.

Vallier, L., Reynolds, D., Pedersen, R.A., 2004. Nodal inhibits differentiation of human embryonic stem cells along the neuroectodermal default pathway. Dev. Biol. 275, 403–421.

Vazin, T., Chen, J., Lee, C.T., Amable, R., Freed, W.J., 2008. Assessment of stromal-derived inducing activity in the generation of dopaminergic neurons from human embryonic stem cells. Stem Cells 26, 1517–1525.

Vescovi, A.L., Gritti, A., Galli, R., Parati, E.A., 1999. Isolation and intracerebral grafting of nontransformed multipotential embryonic human CNS stem cells. J. Neurotrauma 16, 689–693.

Vrana, K.E., Hipp, J.D., Goss, A.M., McCool, B.A., Riddle, D.R., Walker, S.J., Wettstein, P.J., Studer, L.P., Tabar, V., Cunniff, K., Chapman, K., Vilner, L., West, M.D., Grant, K.A., Cibelli, J.B., 2003. Nonhuman primate parthenogenetic stem cells. Proc. Natl. Acad. Sci. USA 100 (Suppl. 1), 11911–11916.

Wang, L., Li, L., Menendez, P., Cerdan, C., Bhatia, M., 2005. Human embryonic stem cells maintained in the absence of mouse embryonic fibroblasts or conditioned media are capable of hematopoietic development. Blood 105, 4598–4603.

Watanabe, K., Kamiya, D., Nishiyama, A., Katayama, T., Nozaki, S., Kawasaki, H., Watanabe, Y., Mizuseki, K., Sasai, Y., 2005. Directed differentiation of telencephalic precursors from embryonic stem cells. Nat. Neurosci. 8, 288–296.

Werbowetski-Ogilvie, T.E., Bosse, M., Stewart, M., Schnerch, A., Ramos-Mejia, V., Rouleau, A., Wynder, T., Smith, M.J., Dingwall, S., Carter, T., Williams, C., Harris, C., Dolling, J., Wynder, C., Boreham, D., Bhatia, M., 2009. Characterization of human embryonic stem cells with features of neoplastic progression. Nat. Biotechnol. 27, 91–97.

Please cite this article as: Wianny, F., et al., Embryonic stem cells in non-human primates: An overview of neural differentiation potential. Differentiation (2011), doi:10.1016/j.diff.2011.01.008

ARTICLE IN PRESS

Wianny, F., Bernat, A., Huissoud, C., Marcy, G., Markossian, S., Cortay, V., Giroud, P., Leviel, V., Kennedy, H., Savatier, P., Dehay, C., 2008. Derivation and cloning of a novel rhesus embryonic stem cell line stably expressing tau-green fluorescent protein. Stem Cells 26, 1444–1453.

Wilson, S.I., Edlund, T., 2001. Neural induction: toward a unifying mechanism. Nat. Neurosci. 4 (Suppl.), 1161–1168.

Wolf, D.P., Kuo, H.C., Pau, K.Y., Lester, L., 2004. Progress with nonhuman primate embryonic stem cells. Biol. Reprod. 71, 1766–1771.

Wu, K., Yang, Y., Wang, C., Davoli, M.A., D'Amico, M., Li, A., Cveklova, K., Kozmik, Z., Lisanti, M.P., Russell, R.G., Cvekl, A., Pestell, R.G., 2003. DACH1 inhibits transforming growth factor-beta signaling through binding Smad4. J. Biol. Chem. 278, 51673–51684.

Xiao, L., Yuan, X., Sharkis, S.J., 2006. Activin A maintains self-renewal and regulates fibroblast growth factor, Wnt, and bone morphogenic protein pathways in human embryonic stem cells. Stem Cells 24, 1476–1486.

Xu, C., Inokuma, M.S., Denham, J., Golds, K., Kundu, P., Gold, J.D., Carpenter, M.K., 2001. Feeder-free growth of undifferentiated human embryonic stem cells. Nat. Biotechnol. 19, 971–974.

Xu, R.H., Peck, R.M., Li, D.S., Feng, X., Ludwig, T., Thomson, J.A., 2005. Basic FGF and suppression of BMP signaling sustain undifferentiated proliferation of human ES cells. Nat. Methods 2, 185–190.

Xu, R.H., Sampsell-Barron, T.L., Gu, F., Root, S., Peck, R.M., Pan, G., Yu, J., Antosiewicz-Bourget, J., Tian, S., Stewart, R., Thomson, J.A., 2008. NANOG is a direct target of TGFbeta/activin-mediated SMAD signaling in human ESCs. Cell Stem Cell 3, 196–206.

Yan, Y., Yang, D., Zarnowska, E.D., Du, Z., Werbel, B., Valliere, C., Pearce, R.A., Thomson, J.A., Zhang, S.C., 2005. Directed differentiation of dopaminergic neuronal subtypes from human embryonic stem cells. Stem Cells 23, 781–790.

Yang, D., Zhang, Z.J., Oldenburg, M., Ayala, M., Zhang, S.C., 2008. Human embryonic stem cell-derived dopaminergic neurons reverse functional deficit in Parkinsonian rats. Stem Cells 26, 55–63.

Yoshimatsu, T., Kawaguchi, D., Oishi, K., Takeda, K., Akira, S., Masuyama, N., Gotoh, Y., 2006. Non-cell-autonomous action of STAT3 in maintenance of neural precursor cells in the mouse neocortex. Development 133, 2553–2563.

Zeng, X., Cai, J., Chen, J., Luo, Y., You, Z.B., Fotter, E., Wang, Y., Harvey, B., Miura, T., Backman, C., Chen, G.J., Rao, M.S., Freed, W.J., 2004. Dopaminergic differentiation of human embryonic stem cells. Stem Cells 22, 925–940.

Zhang, S.C., 2006. Neural subtype specification from embryonic stem cells. Brain Pathol. 16, 132–142.

Zhang, S.C., Wernig, M., Duncan, I.D., Brustle, O., Thomson, J.A., 2001. In vitro differentiation of transplantable neural precursors from human embryonic stem cells. Nat. Biotechnol. 19, 1129–1133.

Zhang, X., Huang, C.T., Chen, J., Pankratz, M.T., Xi, J., Li, J., Yang, Y., Lavaute, T.M., Li, X.J., Ayala, M., Bondarenko, G.I., Du, Z.W., Jin, Y., Golos, T.G., Zhang, S.C., 2010. Pax6 is a human neuroectoderm cell fate determinant. Cell Stem Cell 7, 90–100.

Please cite this article as: Wianny, F., et al., Embryonic stem cells in non-human primates: An overview of neural differentiation potential. Differentiation (2011), doi:10.1016/j.diff.2011.01.008