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Human foetal brain tissue as quality control when developing stem cells towards cell replacement therapy for neurological diseases

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Human foetal brain tissue has been used in experimental and clinical trials to develop cell replacement therapy in neurodegenerative disorders such as Parkinson's disease and Huntington's disease. These pioneering clinical studies have shown proof of principle that cell replacement therapy can be effective and is worthwhile to develop as a therapeutic strategy for repairing the damaged brain [1-5]. However, because of the limited availability of foetal brain material, and difficulties in producing standardized and quality-tested cell preparations from this source, there have been extensive efforts in investigating the potential use of alternative cell sources for generating a large number of transplantable, authentic neural progenitors and neurons. In this review we highlight the value of using human foetal tissue as reference material for quality control of acquired cell fate of in-vitro generated neurons pretransplantation and post-transplantation.
Keywords:
cell replacement therapy, human foetal tissue, Huntington's disease, neural progenitors, neurological diseases, Parkinson's disease, stem cells

INTRODUCTION

Since the first report of human embryonic stem cells [6], the stem cell field has made significant advances when it comes to establishing, culturing and differentiating human pluripotent stem cells into defined lineages, including subtype-specific neurons. Knowledge obtained from developmental studies have provided tools to manipulate stem cells into becoming specific neural cell types, that express distinct subsets of genes/proteins, have similar morphology and act in a way comparable with their in-vivo counterparts [7–11]. With the advancement of cellular reprogramming; induced pluripotent stem cells [9,12–15] induced neural progenitors [16,17] and induced neurons [18,19] are additional future sources of transplantable neurons on the horizon.

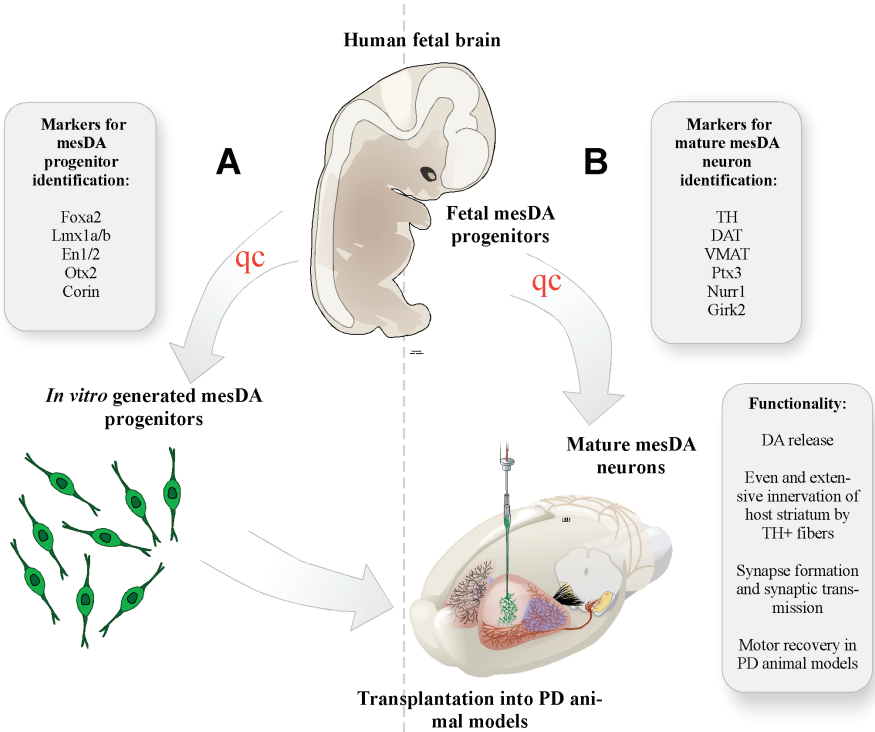

Fig. 1 Human foetal tissue for quality control (qc) of in-vitro generated cells pretransplantation and post-transplantation. Several markers for mesencephalic dopamine (mesDA) progenitors have been validated on human foetal tissue and can be used to validate the quality of protocols aiming at generating mesDA progenitors in vitro (a). Moreover, transplanted foetal mesDA progenitors can serve as a quality control (qc) of in-vitro generated mesDA progenitors post-transplantation, in terms of expected expression of mature mesDA markers, integration into the host tissue and functional recovery in Parkinson's disease animal models (b).

To use any of these renewable sources of cells in the clinic, fully characterized and safe cell preparations produced under Good Manufacturing Practice conditions is a necessity. In addition, the authenticity and functionality of the stem cell-derived human mesencephalic dopamine (mesDA) neurons needs to be fully established in vitro as well as in vivo, in animal models. We argue that human foetal tissue material serve as a valuable resource and provides a 'golden standard' comparison when validating antibody specificity, studying the phenotype of the resulting cells in vitro and predicting the capacity of in-vitro generated cells upon transplantation (Fig. 1).

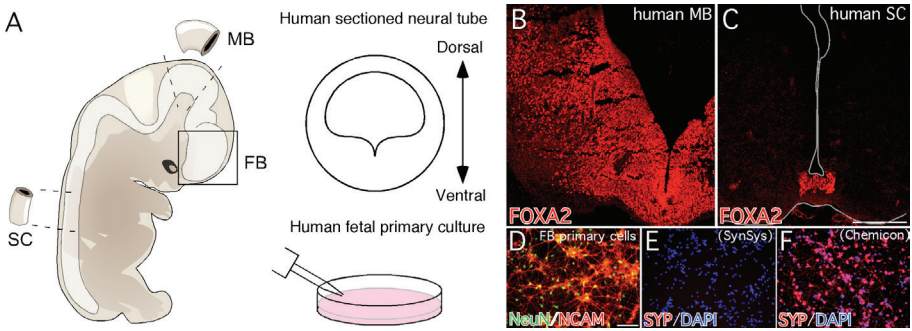

Fig. 2 Validating antibody specificity on human foetal tissue and cells. Human foetal tissue from the spinal cord (SC), midbrain (MB) and forebrain (FB) was dissected out for sectioning and/or establishing primary cultures (a). A FOXA2 antibody (sc-6554) was tested for proper labelling of the human floor plate and was successfully seen to label only the cells of the ventral neural tube in MB as well as SC (b, c). Human foetal cortical primary cultures from subdissected human FB were used to test the specificity of two different synaptophysin (SYN) antibodies, previously reported to recognize SYN protein in neurons of mice [20,21]. The neurons labelled with NeuN and NCAM (d) were not recognized by the SYN ab from Synaptic Systems (10111) (e), but by the mouse anti SYN from Chemicon (MAB329) (f). Scale bars=100 μm.

Quality control of in-vitro generated cells

As new protocols for the generation of subtype-specific neurons are devised, it is very important to analyse the quality and determine the authenticity of these cells. In Parkinson's disease, loss of mesDA neurons leads to the major motor symptoms that are characteristic of Parkinson's disease. The knowledge that cells isolated from human foetal midbrain can provide efficient relief in cell replacement therapy for Parkinson's disease, and the possibility to collect limited numbers of human foetal brain cells from terminations of pregnancy, presents the opportunity to use these cells as a 'gold standard' for direct comparison with any cells generated in a culture dish (Fig. 1a).

One common way to characterize the phenotype of subtype-specific neurons and neural progenitors generated in vitro is to analyse the gene and protein expression of these cells and compare this to their in-vivo counterparts. When working with human cells, protein expression analysis presents particular challenges.

Species	Antibody	Working dilution	Company	order no.
rb	BLBP	1:5000	Chemicon	AB9558
m	Brain3a	1:50	Santa Cruz	sc-8429
m	En-1	1:100	Hybridoma bank	4G11-s
g	Foxa2	1:600	Santa Cruz	sc-6554
rb	GFAP	1:1000	DAKO	Z0334
m	Isl1	1:100	Hybridoma bank	39-405
rb	LRP4	1:100	Orbigen	PAB10777
m	Map2	1:250	Sigma	M1406
m	Mash1	1:500	BDPharMingen	556604
m	Nestin	1:200	BD Bioscience	611658
g	Ngn2	1:100	Santa Cruz	sc-19233
m	Nkx6.1 (IgG1)	1:100	Hybridoma Bank	F55A10
rb	Nurr1	1:1000	Santa Cruz	sc-990
rb	Pax6	1:500	BioSite	PRB278P
m	Sox2	1:50	R&D	MAB2018
rb	TH	1:2000	Chemicon	AB152
m	TH	1:1000	Chemicon	MAB318
ch	vimentin	1:10000	Chemicon	AB5733
g	vimentin	1:1000	Sigma	V4630
m	ph-Vimentin (4A4)	1:2000	MBL	D076-3
rb	5-HT	1:10000	IncStar	20080
rb	Lmx1a	1:2000	Millipore	AB10533
m	Oct3/4	not tested	Chemicon	AB9558
rat	Corin	1:200	R&D Systems	MAB2209
rb	GABA	1:1000	Sigma	A2052
m	HuNuc	1:200	Millipore	MAB1281
g	Otx2	1:1000	Neuromic	GT15095
g	Sox17	not tested	R&D Systems	MAB1924
rb	Brachury	not tested	Abcam	ab20680
rb	Sox1	1:100	Cellsignaling	41945

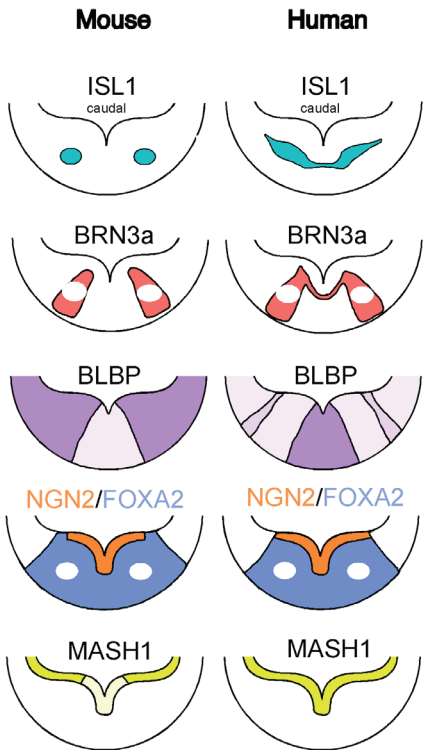

Fig. 3 Specie differences observed between mouse and human. Schematic illustration of some species differences between mouse and human observed in our comparative studies on the human and mouse ventral midbrain [24,25].

Firstly, numerous antibodies directed against specific proteins are commercially available and have been extensively tested and used in developmental studies of chicks and mice for decades. However, the specificity of the majority of these antibodies has not been validated on human brain tissue. The direct use of these antibodies on human cells can, therefore, give misleading data, by providing false positive or false negative results. In our lab, we test the specificity of antibodies on sections from different regions of the human foetal brain, as well as in primary culture of human foetal brain cells (Fig. 2). To date, we have validated 70 antibodies on human tissue and found that less than 40% of these reliably label the correct cell population when tested on human embryos or human primary cultures. We here provide a list of antibodies that we have validated and found to be specific in sections of human foetal brain tissue (Table 1).

Secondly, although there are extensive similarities in the expression pattern of genes and proteins between species, differences have been observed between rodents and humans [22–25]. Differences observed in our studies are summarized in Fig. 3. For example, in the murine ventral midbrain (VM), brain lipid binding protein (BLBP) is highly expressed by radial glial cells positioned lateral to the mesDA neuron progenitor domain. In human, BLBP shows the opposite expression pattern, being highly expressed within the mesDA neuron progenitor domain but absent from the lateral cell population [25]. Species variation was also observed in the distribution of the proneural gene, Neurogenin 2 (NGN2) expressing cells in the ventricular zone of the VM, but not in the spinal cord [24]. Thus, the possible species difference in the temporal and spatial gene and protein expression is an important aspect to be aware of and to consider when analysing the identity of in-vitro generated cells.

In-vivo analysis

Human foetal cells also serve as a valuable and necessary reference material when analysing in-vitro generated cells after transplantation (Fig. 1b). Human cells mature slowly in vivo [26,27] and thus long time points are needed for complete assessment, but xenotransplantation in rodent models are connected with concerns of immunosuppression to ensure graft survival. This can be addressed in several ways, including the use of athymic nude rats who have a compromised immune system because of a lack of T cells; or the use of standard rat breeds, such as Sprague–Dawley, but to immunosuppress them with daily intraperitoneal injections of ciclosporin A. Daily immunosuppression can, however, only be maintained for a certain time before the animal begins to suffer side effects from the drug treatment and/or the administration procedure, in our personal experience 18 weeks being the longest optimal time. There are few studies where a time course of improvement of lesion-induced behavioural deficits following transplantation of human foetal VM has been performed. Brundin et al. [28] observed a reduction in amphetamine-induced rotation after only 15 weeks post-transplantation, similarly Rath et al. [29] observed the same effects after 14 weeks. In our study using hESC-derived mesDA neurons we observed a significant reduction in rotations that continued to improve from 12 to 16 weeks post-transplantation [7]. Thus using a model with daily immunosuppression allows us to observe the initial phase of graft-induced behavioural recovery mediated by human neurons in rodent models of Parkinson's disease. If more long-term studies need to be performed then athymic nude rats become more attractive, but they are surrounded with cost, logistic and practical issues regarding their housing, handling and experimental use. Behavioural assessments using nude rats are not well established so some initial caution would be advised when initiating studies using such strains.

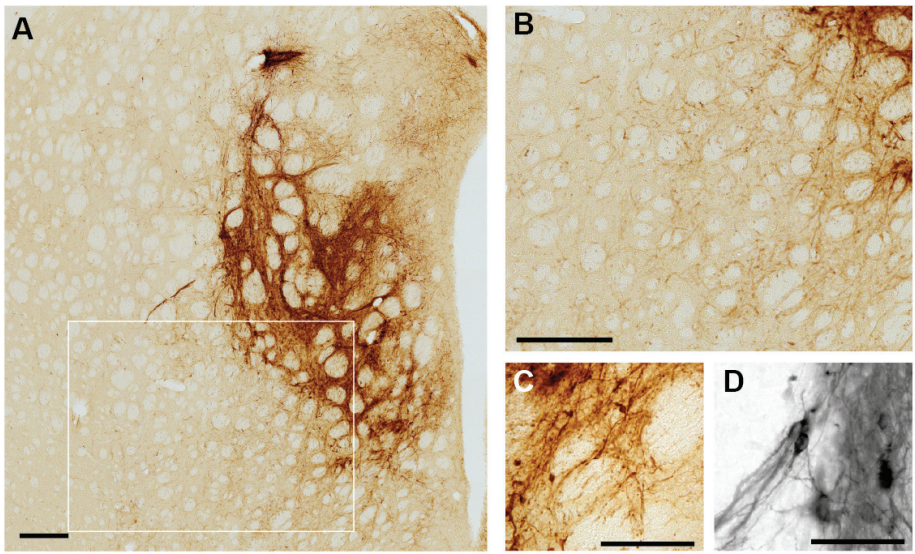

Fig. 4 Predictive Quality Control after transplanatation. Photomicrographs of sections immunostained for tyrosine hydroxylase (TH) depict a transplant of human foetal ventral midbrain 4 weeks post-transplantation (a). This graft has good survival and axonal outgrowth into the surrounding 6-hydroxydopamine lesioned host striatum (b). High magnification reveals a mixture of more mature dopamine neurons with a large angular soma, and more immature ones with small round somas and stunted primary axons (c). After 24 weeks survival in athymic nude rats, human dopamine neurons display a more typical mature morphology (d). Scale bars: (a, b)=250 μm; (c, d)=100 μm.

Because human foetal cells are known to function both in animal models as well as in clinical trials, human foetal VM tissue can serve as a valuable predictive control for the in-vitro generated mesDA-patterned cells in terms of what to expect from survival, neuronal composition and innervation capacity also at early time points when behavioural recovery cannot be measured. In Fig. 4 we depict a transplant of a 6 week human foetal VM 4 weeks post-transplantation (Fig. 4a). Upon close inspection, significant axonal extension and innervation can be observed into the host striatum despite most neurons still having an immature neuronal morphology (Fig. 4b and c). We also highlight that long-term analysis, 24 weeks post-transplantation, is needed to observe mature neuronal morphology characteristic of mesDA neurons (Fig. 4d).

In our experience, cells that have similar phenotype in terms of morphology, innervation and protein expression to primary foetal cells after 6 weeks are likely also

to become functional when left to mature long term. Thus, short-term comparative analysis serves as a prediction of long-term differentiation and function. In a recent study, we performed a side-by-side analysis of grafted human foetal and hESC-derived mesDA neurons in terms of expression of regional markers, morphology and innervation 6 weeks post-transplantation. At this time point, dopamine neurons derived from hESCs were indistinguishable from those derived from foetal VM. Both sources of mesDA neurons exhibited coexpression of mesDA markers such as FOXA2, LMX1A, EN-1 and NURR1, had mature neuronal morphology and innervated similar host structures. The hESC-derived neurons maintained their acquired in-vitro regional identity post-transplantation, and terminally differentiated into functional mesDA neurons after 18 weeks [7]. Similar observations of markers of mesDA regional identity have been made using similar in-vitro protocols [9,30].

In summary, human foetal tissue has in many aspects been shown to serve as an important source of material for quality control of in-vitro generated cells. Because of the slow maturation of human cells in vitro and in vivo, full confirmation of authenticity and function in vitro and in animal models is not possible. A side-by-side comparison with foetal cells known to be able to recover motor deficits in Parkinson's disease patients, may be the best way to predict clinical performance and decide on when to move forward to the first in man trials.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

[1] Lindvall O., Developing dopaminergic cell therapy for Parkinson's disease – give up or move forward? Mov Disord 2013; 28(3):268-273.

[2] Benraiss, A., SA Goldman Cellular therapy and induced neuronal replacement for Huntington's disease. Neurotherapeutics 2011; 8(4):577-590.

[3] Lundvall O., et al. Grafts of fetal dopamine neurons survive and improve motor function in Parkinson's disease. Science 1990; 247(4942):574-577.

[4] Barry, DI et al., Grafts of fetal locus coeruleus neurons in rat amygdala-piriform cortex suppress seizure development in hippocampal kindling. Exp Neurol 1989; 106(2):125-132.

[5] Lindvall, O. et al. Transplantation of fetal dopamine neurons in Parkinson's disease: one-year clinical and neurophysiological observations in two patients with putaminal implants. Ann Neurol 1992; 31(2):155-165.
[6] Thimson, JA et al. Embryonic stem cell lines derived from human blastocysts. Science 1998; 282(5391):1145-1147.
[7] Kirkeby, A et al. Generation of regionally specified neural progenitors and functional neurons from human embryonic stem cells under defined conditions. Cell Rep 2012; 1(6):703-714.
[8] Ma, L et al. Human embryonic stem cell-derived GABA neurons correct locomotion deficits in quinolinic acid-lesioned mice. Cell Stem Cell 2012; 10(4):455-464.
[9] Kriks, S et al. Dopamine neurons derived from human ES cells efficiently engraft in animal models of Parkinson's disease. Nature 2011; 480(7378):547-551.
[10] Carri, AD et al. Developmentally coordinated extrinsic signals drive human pluripotent stem cell differentiation toward authentic DARPP-32+ medium-sized spiny neurons. Development 2013; 140(2):301-312.
[11] Maroof, AM et al. Directed differentiation and functional maturation of cortical interneurons from human embryonic stem cells. Cell Stem Cell 2013; 12(5):559-572.
[12] Liu, Y et al.Directed differentiation of forebrain GABA interneurons from human pluripotent stem cells. Nat Protoc 2013; 8(9):1670-1679.
[13] Xi, J et al. Specification of midbrain dopamine neurons from primate pluripotent stem cells. Stem Cells 2012; 30(8):1655-1663.
[14] Wernig, M et al. Neurons derived from reprogrammed fibroblasts functionally integrate into the fetal brain and improve symptoms of rats with Parkinson's disease. Proc Natl Acad Sci USA 2008; 105(15):5856-5861.
[15] Takahashi, K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell 2006; 126(4):663-676.
[16] Kim, J et al. Direct reprogramming of mouse fibroblasts to neural progenitors. Proc Natl Acad Sci USA 2011; 108(19):7838-7843.
[17] Ring, KL et al. Direct reprogramming of mouse and human fibroblasts into multipotent neural stem cells with a single factor. Cell Stem Cell 2012; 11(1):100-109.
[18] Vierbuchen, T et al. Direct conversion of fibroblasts to functional neurons by defined factors. Nature 2010; 463(7284):1035-1041.
[19] Pfisterer, U et al. Direct conversion of human fibroblasts to dopaminergic neurons. Proc Natl Acad Sci USA 2011. 108(25):10343-10348.

[20] Priller, C et al. Synapse formation and function is modulated by the amyloid precursor protein. J Neurosci 2006; 26(27):7212-7221.
[21] Zhang, XM et al. Beta-secretase-1 elevation in transgenic mouse models of Alzheimer's disease is associated with synaptic/axonal pathology and amyloidogenesis: implications for neuritic plaque development. Eur J Neurosci 2009; 30(12):2271-2283.
[22] Fourgerousse, F et al. Human-mouse differences in the embryonic expression patterns of developmental control genes and disease genes. Hum Mol Genet 2000; 9(2):165-173.
[23] Mestas, J, Hughes, CC Of mice and not men: differences between mouse and human immunology. J Immunol 2004; 172(5):2731-2738.
[24] Nelander, J, Hebsgaard, JB, Parmar M Organization of the human embryonic ventral mesencephalon. Gene Expr Patterns 2009; 9(8):555-561.
[25] Hebsgaars, JB et al. Dopamine neuron precursors within the developing human mesencephalon show radial glial characteristics. Glia 2009; 57(15):1648-1658.
[26] Stromberg, I et al. Long-term effects of human-to-rat mesencephalic xenografts on rotational behavior, striatal dopamine receptor binding, and mRNA levels. Brain Res Bull 1995; 38(3):221-233.
[27] Belkadi, AM et al. Maturation of fetal human neural xenografts in the adult rat brain. Exp Neurol 1997; 144(2):369-380.
[28] Beundin P et al. Behavioural effects of human fetal dopamine neurons grafted in a rat model of Parkinson's disease. Exp Brain Res 1986; 65(1):235-240.
[29] Rath, A et al. Survival and functional restoration of human fetal ventral mesencephalon following transplantation in a rat model of Parkinson's disease. Cell Transplant 2013; 22(7):1281-1293.
[30] Maria, S et al. Improved cell therapy protocol for Parkinson's disease based on differentiation efficiency and safety of hESC-, hiPSC and non-human primate iPSC-derived DA neurons. Stem Cells 2013; 31:1548–1562.