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Commentary

# Transplantable midbrain dopamine neurons: A moving target

J. Carlos Villaescusa, Ernest Arenas *

*Laboratory of Molecular Neurobiology, Department of Medical Biochemistry and Biophysics, Karolinska Institute, S-171 77, Stockholm, Sweden*

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Parkinson's disease (PD) is a progressive neurodegenerative movement disorder, characterized by a loss of dopaminergic (DA) neurons in the midbrain. Current therapies offer temporary symptomatic relief, but do not modify the course of disease. Alternative therapeutic strategies aiming at changing the course of disease have been developed over the last years. Amongst them, cell replacement therapy has been considered as a promising alternative. Grafts of fetal ventral mesencephalic tissue have been found to ameliorate the symptoms and change the course of disease in some Parkinsonian patients, but the limited availability of human tissue and the difficulties to standardize their quality have led to the search of novel cell sources. Pluripotent stem cells are nowadays considered as ideal tools for cell replacement therapy because they are highly expandable *in vitro*, and standardized protocols to control their differentiation are being developed. However, despite substantial progress during recent years, translation into an *in vivo* setting has been challenging. Indeed, transplantable DA neurons need to be correctly specified and differentiated in order to obtain appropriate innervation of the striatum and avoid the risks of graft overgrowth or cell death. It has been previously reported that nigral A9, but not ventral tegmental area A10 DA neurons, are able to re-innervate the host striatum and are thus considered the desired cell type for transplantation (Thompson et al., 2005). However, previous studies using whole or partially dissected ventral midbrain (VM) tissue have not been able to identify the cell type that was responsible for the therapeutic effect observed after grafting. DA neurons themselves have been thought to be the desired cell type and, along this line of thought, fluorescence-activated cell sorting (FACS sorting) has been used to select for DA neurons and to avoid unwanted cells (Pruszak et al., 2007). Interestingly, transplantable DA neurons have been obtained from fractions isolated from Pitx3-GFP embryonic stem (ES) cells (Hedlund et al., 2008), or polysialic acid-neural cell adhesion molecule (PSA-NCAM) positive cells derived from Lmx1a overexpressing ES cells (Friling et al., 2009). However, within the DA lineage, the optimal developmental stage, phenotype and cell type needed for transplantation remain to be determined. A recent study (Jonsson et al., 2009) describes the first systematic transplantation analysis of FACS-sorted cells isolated from the developing midbrain at different stages of development. In this publication, Jonsson et al. (2009) investigated the capacity of DA progenitors, precursors and neurons, isolated at distinct stages of midbrain development, to survive, differentiate and induce functional behavioral recovery after transplantation. The first interesting conclusion from that study is that DA neurons are not the best cell type for transplantation, as their survival was poor. Second, the optimal cell type for transplantation was surprisingly found in different VM compartments, at distinct developmental stages, and therefore expressed distinct markers. At E10.5, lateral floor plate (FP) ventricular zone (VZ) progenitors gave rise to A9 *substantia nigra* (SN) DA neurons, but survived poorly. In contrast, at E12.5, intermediate zone (IZ) postmitotic migratory precursors were the most important source of transplantable neurons, with regard to survival and functional recovery, but gave rise to both A9 and A10 neurons. This study thus underscores that the source of optimal transplantable DA neurons in the developing midbrain changes from one compartment and cell type to another (VZ progenitor to IZ precursor). These findings shed light on the important issue of identifying and isolating transplantable cells capable of giving rise to midbrain DA (mDA) neurons, and particularly, to DA neurons of the SN (A9) subtype. Overall, the study by Jonsson et al. (2009) provides important tools for the isolation of transplantable populations. In this review, we discuss the important implications of these findings for current efforts to develop well-characterized stem cell-derived mDA neurons for cell replacement therapy, as well as future avenues.

*Abbreviations:* mDA, midbrain dopaminergic; VZ, ventricular zone; IZ, intermediate zone; MZ, mantle zone; VTA, ventral tegmental area; SN, *substantia nigra*; Nurr1, nurr77-related receptor 1; PD, Parkinson's disease; TH, tyrosine hydroxylase; VM, ventral mesencephalon; Sox2, sex determining region Y-box2; Lmx1a, LIM homeobox transcription factor 1 alpha; BAF, Boc-Asp(OMe) fluoromethyl ketone; Pitx3, paired-like homeodomain transcription factor 3; ALDH, Aldehyde dehydrogenase.

* Corresponding author.
*E-mail address:* ernest.arenas@ki.se (E. Arenas).

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*Transplantable sources of DA neurons during early midbrain development.* At E10.5, during early mouse DA neurogenesis, the VZ of the VM is mainly composed by radial glia-like progenitor cells. These cells express the transcription factor Sox2 (sex determining region Y-box2), important for the regulation of embryonic and neural development. At this stage, DA neuron progenitors in the medial and the lateral FP also express the homeodomain transcription factor Lmx1a (LIM homeobox transcription factor 1 alpha). In the medial FP, radial glia-like progenitor cells express high levels of the transmembrane serine protease Corin (Ono et al., 2007). Radial glia-like cells in the FP divide to generate postmitotic daughter cells, DA neuroblasts or DA precursors (Bonilla et al., 2008), which at E10.5 are mainly located within the lateral IZ and they are characterized by the expression of the transcription factor Nurr1 (Nuclear receptor related 1). Three compartments can thus be observed at E10.5: the medial FP (Corin$^{high}$, Lmx1a$^{+}$, VZ), the lateral FP (Corin$^{low}$, Lmx1a$^{+}$ VZ), and the lateral IZ (Lmx1a$^{+}$, Nurr1$^{+}$) (Fig. 1A). Jonsson et al. (2009) employed two strategies to FACS fractions from the developing VM at E10.5 for transplantation, the Neurogenin (Ngn) 2-GFP mouse (Seibt et al., 2003) and Corin antibodies (Ono et al., 2007). Since Ngn2-GFP$^{+}$ cells were only found in the lateral FP, the Ngn2-GFP mouse was used to isolate Ngn2-GFP$^{low/neg}$ FP cells, and Corin antibodies to select Corin$^{high}$ medial FP VZ cells. Four distinct populations were thus isolated and used for transplantation. The first one consisted of Ngn2-GFP$^{high}$; the second was Ngn2-GFP$^{low/neg}$ FP cells; the third consisted of Corin$^{high}$ medial FP radial-glia-like cells; and the fourth population of Corin$^{low/negative}$ containing lateral FP VZ progenitors, IZ precursors, and possibly basal plate cells included in the dissection (Fig. 1A). Transplantation of these four populations of E10.5 cells into the dopamine-depleted striatum produced several interestingly results.

Transplantation of Ngn2-GFP$^{high}$ progenitors (basal plate cells, lateral to Lmx1a domain) generated few mDA neurons while the Ngn2-GFP$^{low/neg}$ cell fraction (FP) yielded more DA neurons (Fig. 1A) and behavioral improvements. Corin-sorted cells (high or low) survived transplantation poorly and failed to induce behavioral benefits. However, these experiments lead to one of the most interesting results in this study. While Corin$^{low/neg}$ gave rise to only Girk2$^{+}$ A9 SN DA neurons, the predominant cell type lost in PD, Corin$^{high}$ cells generated both A9 and A10 DA neurons. These findings show an anatomically distinct origin of A9 SN DA neurons, identify Corin$^{low/neg}$ as a promising marker to isolate E10.5 A9 mDA progenitors and suggest that future cell sorting strategies should aim at improving the isolation of lateral FP VZ A9 mDA progenitors.

*Transplantable sources of DA neurons during mid-neurogenesis in the developing midbrain.* To date, most transplantation studies have focused on the isolation of VM cells at E12.5 because this is the period when DA neurogenesis peaks and before extensive DA neurite growth. At a histological level, an expansion of VZ progenitors and postmitotic cells of the IZ and mantle zone (MZ) in the VM FP becomes apparent. While at E10.5, only few DA neuroblasts were observed in the IZ, at E12.5, this layer consists of two lateral "mountains" of Nurr1$^{+}$ cells connected by a thin medial group of Nurr1$^{+}$ cells. These cells migrate to reach the MZ and start expressing tyrosine hydroxylase (TH), as well as the Paired-like homeodomain transcription factor 3, Pitx3 (Smidt et al., 1997). At this stage, four compartments can thus be observed: the medial VZ FP (Lmx1a$^{+}$, Corin$^{high}$), the lateral VZ FP (Corin$^{low}$, Lmx1a$^{+}$), the IZ (Lmx1a$^{+}$, Nurr1$^{+}$) and the MZ (Lmx1a$^{+}$, Nurr1$^{+}$, Pitx3$^{+}$, TH$^{+}$) (Fig. 1B).

At E12.5, Jonsson et al. (2009) again used Corin antibodies as well as Ngn2-GFP and Pitx3-GFP expression to isolate and characterize the suitability of distinct cell populations within the VM FP for transplantation. Contrary to E10.5 derived cells, at E12.5 Ngn2-GFP$^{high}$ cells were found in the FP (particularly the IZ, but also the MZ, basal part of the VZ and along the VZ–IZ boundary in the FP and BP, Fig. 1B). Jonsson et al. (2009) took advantage of this expression pattern to FACS sort Ngn2-GFP$^{high}$ (rich in Nurr1$^{+}$ mDA precursors of the IZ FP), or Ngn2-GFP$^{low/neg}$ cells, that included E12.5 progenitors of the VZ and neurons from the MZ, mainly from the FP, but presumably also from the basal plate. Moreover, to determine the contribution of mDA neurons to survival within the grafts, Pitx3-GFP mice were also used (Zhao et al., 2004). While the Pitx3-GFP$^{high}$ fraction only contained TH$^{+}$ cells located in the MZ, the Pitx3-GFP$^{low/neg}$ fraction contained both DA progenitors and precursors of the VZ and IZ (Fig. 1B).

The analysis of transplantable DA cells preparations performed by Jonsson et al. (2009) clearly shows that the most interesting cell for cell replacement therapy, A9 DA neurons, can be obtained from E10.5 lateral FP Corin$^{low/neg}$ VZ cells in the VM, but these cells do not transplant well. Instead cells contained within the IZ of the midbrain FP (Ngn2-GFPhigh fraction at E12.5, and FP Ngn2-GFP$^{low/neg}$ at E10.5) provided the best functional outcome after transplantation. These results thus indicate that the best source of transplantable midbrain DA cells is a moving target, as in the course of development, optimal cells for transplantation are different in terms of the selectable markers they express, and the position they occupy in the VM. This is an important point as strategies for the isolation of transplantable cells derived from ES cell preparations will need to take into account the temporal aspect of development.

*Preventing cell damage and cell loss during cell separation.* The study by Jonsson et al. (2009) also highlights the issue that the survival of cells in the DA lineage, particularly of DA progenitors and DA neurons, needs to be improved in order to enhance the therapeutic potential of sorted cells in cell replacement therapy.

These results indicated that DA neurons (Pitx3-GFP$^{high}$ cells) and medial FP VZ progenitors (Corin$^{high}$ cells) were poor sources of cells for transplantation at E12.5. The negative results obtained with Pitx3-GFP$^{high}$ cells are to some extent surprising, provided previous positive results obtained by Hedlund et al. (2008), who selected ES cell-derived Pitx3-GFP cells by FACS and obtained significant engraftment and behavioral recovery. It should be noted, however, that other than the different cell source, the number of ES cell-derived Pitx3-GFP cells used in that study was greater ($6 \times 10^4$ cells) than that used in the study by Jonsson et al. (2009) ($2 \times 10^4$ cells). Moreover, Hedlund et al. (2008) significantly enhanced the survival of grafted Pitx3-eGFP neurons by using a cocktail of growth factors (BDNF and GDNF) and adding the pan-caspase inhibitor BAF to the cell suspension prior to engraftment. These differences could explain the discrepancy in graft outcomes.

The negative results obtained with Corin$^{high}$ compared to Corin$^{low/neg}$ cells at E12.5 were also surprising. One possibility is that Corin$^{high}$ cells are particularly vulnerable at this stage. However, the cell composition of these two fractions was also very different. While the Corin$^{high}$ fraction contained only VZ progenitors from the midline, the Corin$^{low/neg}$ contained not only lateral VZ progenitors, but also a large number of precursors and neurons from the IZ and MZ, respectively. It is thus possible that lateral VZ progenitors are as vulnerable as Corin$^{high}$ at E12.5 and that the better result of the Corin$^{low/neg}$ fraction reflects the contribution of IZ precursors, as described for Ngn2-GFP$^{high}$ cells.

Interestingly, Pitx3-GFP$^{high}$ cells isolated from the MZ showed poor survival upon transplantation, indicating that relatively few of the mature mDA neurons survive transplantation. A likely explanation is that cell dissociation and FACS are too traumatic for DA neurons, which already have elaborate axonal processes (Gates et al., 2004). Another explanation could be that these cells lack additional cell extrinsic signals to maintain a proper differentiation of precursors into DA neurons when they are placed in an adult heterotopic environment. Additionally, the selective isolation of DA neurons may affect their survival, as it excludes the trophic support arising from other cell types in the developing midbrain such as glial cells (Sortwell et al., 2000, Takeshima et al., 1994). Similarly, grafts of E10.5 Corin-sorted