COMMENTARY

Sorting the wheat from the chaff in dopamine neuron-based cell therapies

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The field of neuronal transplantation for Parkinson's disease has evolved through a remarkable convergence of ideas and techniques (1, 2). Over the last decade, attempts to replace missing dopamine neurons have evolved from an innovative and complex fetal dopamine cell transplantation method toward a potential scalable method that depends on stem cell-derived dopamine neurons (2–10). For functional recovery to occur, the precise midbrain dopamine neuron (denoted A9) lost—which normally connects exactly with the caudate putamen target and inititates movement—needs to be replaced. A series of transplantation experiments using transgenic mice and elimination of the A9 neuron have documented the need for such specificity (1, 11). Therefore, in future transplantation trials, it will be essential to transfer the active and necessary midbrain dopamine neurons back to the patients.

Identifying the Desirable Cell Type

How is identification of this specific dopamine neuron-type accomplished during normal brain development? The answers lie in the developmental genetic determinants for the midbrain dopamine neurons versus those of other dopamine neurons in the retina, dorsal midbrain, or hypothalamus. The nervous system develops through regional specification and genetic programming. For the midbrain, a series of events that involve gene transcriptional steps needs to take place (12–18). First, for precision region specification, it is important that sonic hedgehog ventralizes the neural tube cellular progenitors. Next, defined regional cell populations proliferate, involving WNT-related molecules and transcription factors, such as Nkx 2.2, Otx 2, and FGF8A/B, to generate the period of expanded neurogenesis. There are further specifications with Engrail gene expression and with master transcription and cell identity genes, such as *Nurr1* and *Pitx3*, which generate the final steps in developmental specification of midbrain neurons into A9 or A10 substantia nigra dopamine neurons (14–17, 19). This developmental molecular machinery is relatively well understood; however, how can it be used to provide a selection of the most relevant therapeutic target cells for Parkinson's disease transplantation?

Cell Sorting for Parkinson's Disease Studies

There are potentially two objectives. One is to eliminate cells that could generate unwanted growth or dilute the target dopamine neurons (20–22). The second opportunity is to enrich for such therapeutic cells using positive selection, which can be accomplished by cell-sorting methods (16, 22). In human embryonic stem cells, it was shown that surface markers that included CD15, CD24, and CD29 could also define specific neural surface biomarker codes for cell sorting using FACS (23). However, more recently it became possible to label and fluorescently detect the expression of marker genes (such as *Nurr1* or *Pitx3*) that indicated specific midbrain dopamine neuron lineages, and this provided a more direct way of sorting and enriching the cells by positive selection (6, 24, 25). Such efforts led to the first evidence that pure (or nearly pure) dopaminergic neural populations could restore function in parkinsonian animal models (6, 24). In particular, Corin expression in the midbrain progenitor cells led to experiments that confirmed that Corin sort selection was an appropriate way to enrich the desirable midbrain dopamine neuron populations (26).

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Neuron and cell-selection studies for clinical applications are proceeding (2). First, Ganat et al. used a GFP reporter-based strategy to identify several molecules that specify the immature midbrain dopamine neurons (6); and Doi al (26) demonstrated that the Corin cell enrichment could provide the needed molecular signature. These authors also identified the selectable surface marker Alcam (26). In a report published in PNAS, Bye et al. (27) demonstrate another strategy that also obtained evidence of at least one of these markers (Alcam). Bye et al. base their analysis on mRNA expression from sorted neurogenin2-GFP progenitor cells (28) and then comparatively by using the GFP^- fraction (nondopaminergic) cell populations expressing Lmx A1 GFP (11), and by post hoc analysis subtracted less-relevant mRNA expression. This arithmetic provided support for a separate analysis and selection of transmembrane proteins expressed only on developing midbrain dopamine neurons. Bye et al. (27) prioritized four surface transmembrane receptor marker candidates: Alcam, Chl1, Gfra1, and Igsf8. The authors confirmed and replicated the findings of Doi et al. (26), that Alcam was expressed widely throughout the midbrain dopaminergic region. Using FACS, Bye et al. (27) were able to sort by the Alcam marker and obtain an almost 30-fold enrichment in midbrain dopamine neurons; however, they lost a large fraction of these cells because of the cell-sorting procedure. When transplanting such enriched cell populations in a rodent model of Parkinson's disease, Bye et al. found that the surviving dopamine neurons had the capacity to reinnervate the host striatal target brain territory and restore function (27).

The neural transplantation field of medicine is moving beyond what has been accomplished previously with the use of bone marrow transplantation of hematopoetic cell lineages (29), which by their nature of continued division are considered stem cells. The success of these new cell therapy methods depends on obtaining scientific support for transplanting live-cell populations that are safe and do not generate unwanted cell populations in

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the patients. It is therefore encouraging to see that many research groups are tackling this practical issue and are providing cell-surface markers, genetic profiles, and direct methods to identify the therapeutic target cells selectively.

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