

# Stem Cell Tracking: Reversing the Silence of the Lambs . . .

**B**oth in the development and decline of an organism, several forms of cell death are pivotal (1,2). If excessive cell death or loss of function occurs in certain areas of an organism, diseases may arise. Neurodegenerative disorders and cardiovascular diseases are common examples, being responsible for a large group of patients associated with a poor quality of life. For some of these diseases the pharmacotherapeutic treatment approaches were markedly improved during the last decades (e.g., the use of L-DOPA and other dopaminergic agents in Parkinson's disease). Still, there is an unmet clinical need for new therapeutic options, since pharmacologic interventions will lose their efficacy on disease progression (3). Moreover, there are many degenerative diseases for which pharmacotherapy is only slightly effective, such as Alzheimer's disease (4) and amyotrophic lateral sclerosis (5).

One of the apparently most straightforward methods for the treatment of diseases caused by cell degeneration is cell replacement therapy. However, finding accurate cell types to serve as a valid replacement for degraded cells appeared to be difficult. Cell therapy has evolved from transfusional medicine, using blood and other products derived from the human body, toward a highly technical, clinical grade approach using thoroughly controlled human cells (6). This, in turn, led to the discovery of many pitfalls, introducing new challenges to be solved.

The main problems related to cell replacement therapy are practical and ethical. Originally, midbrain cells from human fetuses were used for brain transplantation, mainly into the brains of patients with Parkinson's disease. These fetuses had to be of a narrow range of gestational ages. Furthermore, per treated patient, about 8 fetuses were needed to harvest enough neural cells. Hence, the only way to collect enough fetal neural cells would be to obtain them from elective abortions (7). The dependence on several abortions for the possible treatment of one patient is not only controversial but also logistically almost impossible. So, it is obvious that the ethics in connection with fetal neuronal cells are very complex. This explains why the topic is currently high on the political agenda of many countries (8).

The results of the first open-label studies in humans on cell replacement therapy were encouraging. In patients with Parkinson's disease, human fetal mesencephalic tissue was transplanted into the striatum. Moreover, recent studies in these transplanted patients showed survival of the transplanted cells for >10 y and functional reinnervation of the striatal area (9–11). It was shown that basal dopamine levels in the striatum were restored. Generally, the studies showed clinical improvement. For some of the transplanted patients, it was demonstrated that they could stop their L-DOPA medication and substantially regained a better quality of life (11). However, additional information was collected from 2 double-blind, prospective trials comparing fetal cell-transplanted patients with sham-operated patients (9,12,13). These studies failed to show a significant improvement in the patients who received the transplants. Moreover, the

investigations were complicated by off-medication dyskinesias. Interestingly, it was suggested that a longer time of culturing the fetal neural cells before transplantation improved the clinical outcome for the patient. From these results, the question arises whether the current procedures of cell replacement are adequate (14). Furthermore, on performance of these neural cell transplants in patients, no methods of cell tracking were used, which would enable a more accurate follow-up.

Because of the varying results obtained with the different neural transplants, new searches are undertaken to provide other sources of neuronal cells. Examples of these other sources would be the use of stem cells, xenograft material, or immortalized (stem) cell lines (7). Especially the latter have great reproduction ability. Thus, is likely that the efficacy of production and collection of cells can be improved. Particularly, the use of stem cells would be highly interesting. Stem cells are defined as undifferentiated, pluripotent cells with prolonged self-renewal capacity and the ability to proliferate extensively (6). Stem cells are not only derivable from fetal tissue but also can be collected from bone marrow, brain, and skin of adults. Moreover, it is possible to differentiate nonneural cells into counterparts that are able to produce neurotransmitters such as dopamine.

After transplantation, transplanted stem cells need to be followed to evaluate their anatomic position and functional condition. First, several methods have been developed to track transplanted stem cells. Iron oxide-labeled cells (for MRI) (15), fluorescently labeled cells, and combinations of these techniques (16) were demonstrated to be feasible for

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cell tracking in the host. Recent studies showed that techniques using MRI have the advantage over fluorescent methods by their ability to be used in humans and larger animals. However, disadvantages are the low sensitivity of the method and the limited number of probes available (17). In contrast, methods using radioisotopes combine both the opportunity of imaging humans and larger animals with a larger sensitivity. Like MRI and fluorescent methods, radioactively labeled cells can serve as a tool to track the fate of the transplanted cells. Recently, the latter has been described by Adonai et al. They used  $^{64}\text{Cu}$ -pyruvaldehyde-bis( $N^4$ -methylthiosemicarbazone) to label rat glioma cells (17). Furthermore, the use of reporter gene labeling is a common concept in cell tracking. Suitable genes for this purpose are uncommon for the cell type (18). Regularly used genes are coding regions for, for example, alkaline phosphatase, firefly luciferase, and chloramphenicol acetyltransferase. The corresponding gene product (a protein) can metabolize a substrate into a detectable product (a color, fluorescence, or chemical). Hence, the reporter gene label enables cell detection by an assay (18). The great disadvantage of these tracking methods is the requirement of a biopsy or even animal sacrifice for the performance of cell tracking.

Second, a variety of methods has been and still is to be developed or has to be adapted for cell functionality assessment. Already known examples of the latter are the currently used nuclear techniques such as  $^{123}\text{I}$ -FP-CIT ( $^{123}\text{I}$ -Ioflupane;  $^{123}\text{I}$ -labeled  $N$ - $\omega$ -fluoropropyl-2 $\beta$ -carbomethoxy-3 $\beta$ -[4-iodophenyl]nortropine),  $^{123}\text{I}$ - $\beta$ -CIT (2 $\beta$ -carbomethoxy-3 $\beta$ -[4-iodophenyl]tropine), and  $^{18}\text{F}$ -DOPA ( $^{18}\text{F}$ -6-fluoro-L-DOPA) imaging of dopaminergic neurons (19,20). These can theoretically be used in combination with the tracking methods to evaluate the anatomy of the transplant in relation to its function. For the imaging of cardiac cell function, it has already been shown that meth-

ods such as  $^{99\text{m}}\text{Tc}$ -sestamibi imaging for cardiac metabolic function can be adapted as a functional test for transplanted cells (21).

Kim et al. (22), on pages 305–311 of this issue of *The Journal of Nuclear Medicine*, describe a novel method for cell tracking of neural stem cells. They used a gene labeling method, by transfection of the human sodium/iodide symporter (hNIS) transgene. The gene was transfected in an immortalized fetal stem cell line using a DNA plasmid containing the gene as well as a promoter derived from the cytomegalovirus and a hygromycin resistance gene. The latter gene is added to be able to select all transfected cells, since the raw cell material is incubated with hygromycin, which kills all nontransfected cells. The new, chimeric cell line (F3-hNIS) is able to accumulate  $^{125}\text{I}$  up to 36-fold more than that of the nontransfected cell line. For imaging purposes, the authors use pertechnetate as an “iodomimetic.” Therefore, the property of pertechnetate accumulation in these cells can be used for noninvasive cell tracking in animals and, theoretically, also in humans. However, after prolonged cell culturing, the ability to accumulate iodine or pertechnetate decreases on cell passage to a new culture medium. This means that after the eighth passage the amount of iodine accumulation is decreased to less than twice the amount for normal cells. Thus, imaging will be hampered when transfected cells are kept in culture for a long time after their transfection. The latter is caused by gene silencing, which prevents the expression of the hNIS gene in the cell. Because gene silencing is a regular process in transfected cell lines, some basics behind mechanisms have been unraveled (23). Gene silencing can be caused by epigenetic modulation such as DNA methylation and histone deacetylation (HDAC). These events prevent the cell’s ability of DNA transcription by steric hampering of the process.

The authors reversed the silencing process by incubating (24 h) the stem cells with a DNA-demethylation inhibitor, 5-azacytidine, and an inhibitor of HDAC, trichostatin A. They proved that this treatment is able to increase radioiodine uptake by a maximal 5.3-fold compared with that of nontreated cells. After transplantation, however, tracking the cells turns out to be difficult, as the sensitivity of the image is not yet a strong property. However, the concept of restoring DNA transcription ability for the production of hNIS remains elegant, but the described method demands optimization. From this article, it is already evident that the quality of the images is likely to improve by optimization of the gene silencing reversal. The authors provided an image in which only trichostatin A-treated cells were used for cell tracking purposes. However, showing an image of cells treated with a low dose of trichostatin A and a high dose of 5-azacytidine would have been of more interest. For this combination, a better radioiodine uptake was described than that for the way in which the imaged stem cells were treated. Another point of validation for the use of 5-azacytidine and trichostatin A is their eventual harm to the host, which can be circumvented by washing the cells until none of these substances is present anymore. Furthermore, the method can be validated by gaining evidence that administering the unwashed cells to the host does not harm either the transplant or the host.

A problem that also must be addressed is the use of an immortalized cell line in humans. Of course, the use of these cell lines can be regarded as a tool contributing to the model of stem cell transplants. But it would be of great value to investigate the use of immortalized cell lines as a source of transplant material, despite of the risks. Interestingly, the advent of conditionally immortalized cell lines will probably solve the continuous division complications of their classic counterparts. These cell lines

have been transfected with temperature-sensitive oncogenes, being mutants of common oncogenes such as simian virus 40. Cattaneo and Conti described the development of an embryonic conditionally immortalized striatal cell line (24). These cells require a lower culture temperature (e.g., 33°C) to continue their proliferation. Raising the temperature to 39°C causes the loss of division capacity as well as the restoration of differentiation hallmarks such as the sensitivity for neurotrophic factors. The latter enable transformation of the neural stem cell into neurons and glia cells (24). Hence, the use of conditionally immortalized cell lines could be of great value, if validated for clinical use. They could be of great help, because they combine proliferative properties for the generation of enough cell material with the ability to stop their division at a desired moment.

Naturally, the use of embryonic human stem cells remains controversial. Therefore, other means also should be explored further to circumvent additional ethical discussions and enhance the progress of cell replacement therapy. The big advantage of the use of other than embryonic stem cells is the option of autologous transplant of cell material, thus eliminating immunologic complications. Problems that are to be dealt with are, for instance, the fact that it is not yet possible to convert adult neural stem cells into mid-brain equivalents producing dopamine (9). The latter would enable autologous stem cell transplantation of functional dopaminergic tissue to any patient without many ethical concerns, and, moreover, without the occurrence of graft-versus-host disease. An interesting new solution to generate human neural stem cells was demonstrated very recently by Dezawa et al. (25). They showed that bone marrow stromal cells from human and rat origin could be differentiated into neural-like cells by treatment with trophic factor. The new cell type did not proliferate anymore,

and it was shown that the cells expressed several neuronal markers. Moreover, these cells were transplanted in model rats of Parkinson's disease. Upon examination, a statistically significant improvement of motor function in the transplanted rats was shown, compared with that of a sham-operated control group (25). Still, much work is to be done before the application of stem cells in humans can take place. Nonetheless, it must be stated that the use of differentiated stem cells from the bone marrow is promising and elegant, allowing probably the use of autologous cells for the treatment of the diseased patient. When we are able to achieve this goal properly, cell transplants may provide patients a better quality of life than pharmacotherapeutic solutions.

In conclusion, we offer some suggestions for the required work to enable cell replacement therapy in humans:

- Better criteria for selecting the suitable patients for cell replacement therapy should be defined (14).
- To prevent ethical discussions and immunologic complications, the use of autologous cell material should be advocated to produce cells for therapeutic purposes.
- Good genetic and biochemical characterization of the used cells is needed to prevent any damage to the host.
- To ensure the availability of cell material, the possibility to use conditionally immortalized cell lines should be investigated and validated thoroughly before application in the patients.
- Validation of the use of agents needed for transplant cell pretreatment is required.
- More cell tracking methods are needed to validate any cell transplant procedure.
- Before application in humans, every type of cell transplant

should be tested in adequate animal models.

- Development and validation of cell functionality tests (such as <sup>18</sup>F-DOPA for Parkinson's disease) are required to ensure also functional tracking of the cells. Cell tracking methods, such as the sodium/iodide symporter gene method described by Kim et al. (22), only track viable cells (20). The latter cells are not necessarily functional. Nevertheless, both cell tracking and determination of cell functionality are obligatory for efficacy evaluation of the transplant.

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