# Generation of Transplantable Striatal Projection Neurons from Human ESCs

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**In this issue of *Cell Stem Cell*, Ma et al. (2012) report a differentiation protocol for generating striatal projection neurons from human embryonic stem cells with high efficiency. The cells survive transplantation, reconnect striatal circuitry, and restore motor function in a mouse model of striatal neurodegeneration that mimics the central pathohology of Huntington's disease.**

Cell replacement therapy for neurological disease, where lost neurons are replaced with new ones, critically depends on a readily available, renewable, and bankable source of cells to be used for transplantation. The derivation of human embryonic stem cells (hESCs) raised hope for the creation of an unlimited source of donor cells for neural transplantation therapy (Thomson et al., 1998); however, the hESC field has progressed more slowly than originally anticipated, facing challenges such as asynchronized and uncontrolled hESC differentiation into mixed populations of cells, their propensity for tumor formation after grafting, and their poor in vivo performance (Gögel et al., 2011). In this issue of *Cell Stem Cell*, Ma et al. (2012) report efficient generation of striatal GABAergic projection neurons from hESCs that integrate into host neural circuitry and correct motor deficits in a mouse model of striatal neurodegeneration.

The authors' strategy was to modify a monolayer differentiation protocol that they had previously established to generate anterior neuroectodermal cells (Pankratz et al., 2007). When combining this protocol with timed and dosed delivery of Sonic hedgehog (SHH), or the small molecule purmorphamine, the neural progenitors were efficiently patterned into a ventral forebrain fate with characteristics of cells in the lateral ganglionic eminence, the structure that gives rise to striatal projection neurons during embryonic development (see Figure 1). Subsequent differentiation of these progenitors yielded functional neurons that produced GABA and expressed key markers of striatal projection neurons at a remarkably high frequency. Interestingly, addition of retinoic acid (RA) caudalized the progenitors such that they maintained their GABAergic phenotype but lost their striatal characteristics. When grafted to the lesioned mouse striatum, the forebrain-patterned progenitors, but not the caudalized progenitors, formed a high proportion of cells with functional and morphological properties of striatal neurons. Only the forebrain-patterned neurons, expressing a DARPP-32-positive striatal phenotype, but not the caudalized DARPP-32-negative GABAergic neurons, could correct motor deficits in animal models of neurodegeneration.

Although previous work had established that striatal projection neurons can be obtained from hESCs (Aubry et al., 2008), the protocol described by Zhang and colleagues reflects a significant advancement as it is performed under defined conditions, avoids the use of feeder cells, and produces DARPP-32-positive striatal neurons with much higher efficiency (70%–80% of all cells in the dish, compared with less than 20% in the Aubry et al. study). Perhaps the most significant advancement in the Ma et al. study is the in vivo performance of the cells. When grafted into the excitotoxin model of striatal neurodegeneration, which replicates the central pathology of Huntington's disease, the hESC-derived striatal progenitors showed good survival over a relatively long time frame. Moreover, the grafted cells exhibited an impressively high degree of neuronal differentiation with a high proportion of GABAergic neurons expressing the characteristic striatal marker DARPP-32 (over 50% of all cells in the grafts). The grafted cells were shown to receive inputs from the host brain and project to correct target structures in the host, accompanied by reversal of the motor deficits seen in the excitotoxin-lesioned mice. Importantly, the animals showed no signs of overgrowth or tumor formation, otherwise associated with grafting of pluripotent cells and immature forebrain-patterned cells in particular (Aubry et al., 2008; Roy et al., 2006).

The current study expands on a series of recent advancements in the development of protocols for directed neural differentiation and generation of specific subsets of CNS neurons from hESCs. New protocols for neural induction have resulted in faster, more synchronized, and efficient generation of neural progenitors and neurons from hESCs (Chambers et al., 2009; Pankratz et al., 2007). The current study, as well as another recent report (Kriks et al., 2011), shows that when these neural differentiation protocols are combined with more refined strategies for regionalization of the induced cellular phenotypes based on developmental principles, it is possible to generate functional subtype-specific neurons in large numbers that survive and form neuron-rich and tumor-free grafts after transplantation. Importantly, the striatal neurons generated by Ma et al., and the dopamine neurons generated by Studer and colleagues, were shown to be functionally efficacious in animal models that replicate central neurodegenerative features of Huntington's and Parkinson's disease, respectively.

It should also be noted that recent developments in cellular reprogramming have opened up new avenues for obtaining human neurons for cell therapy and other applications. The ability to reprogram the identity of easily accessible

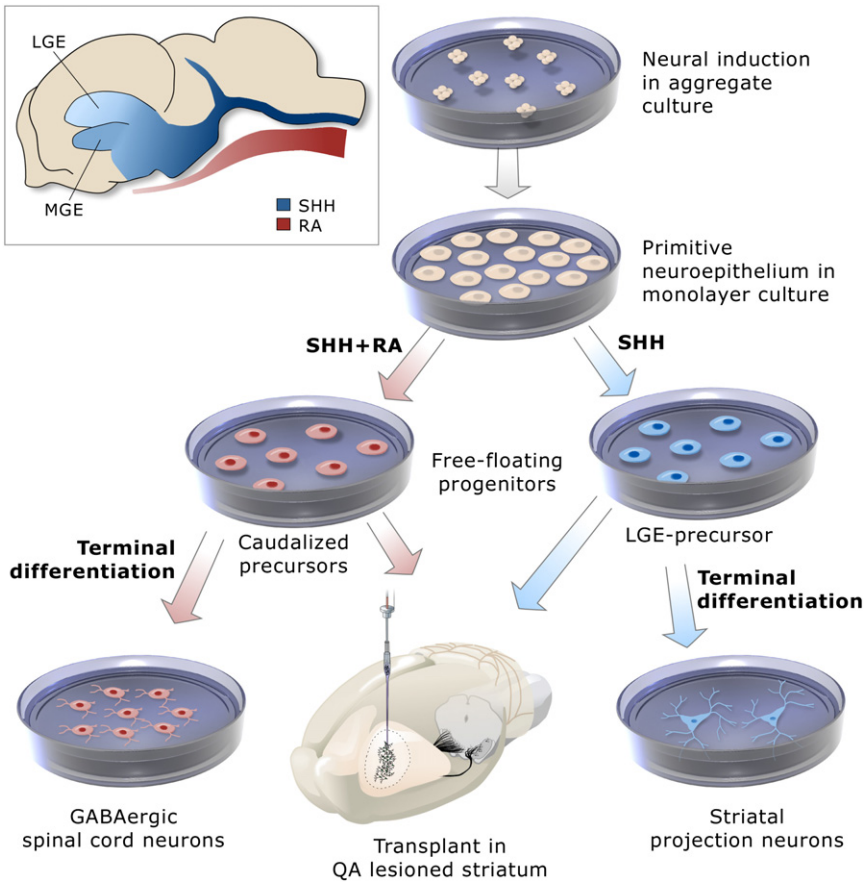


**Figure 1. Application of the Developmental Inductive Signals SHH and RA Enables Differentiation of Defined Subsets of Neurons from hESCs**

Schematic diagram illustrating the expression of Sonic hedgehog (SHH) along the ventral midline (blue) and the gradient of its concentration in the ventral forebrain which was used to give the cells a ventral, striatal identity. The rostro-caudal gradient of the caudalizing factor retinoic acid (RA), used in this study, is shown in red. Primitive neuroepithelial cells were patterned with SHH to generate lateral ganglionic eminence (LGE) progenitors that subsequently differentiated into striatal projection neurons in vitro and after transplantation in vivo. When the neuroepithelial cells were exposed to SHH as well as RA, GABAergic spinal cord neurons were formed. When transplanted to the quinolinic-acid-lesioned striatum, the striatal, but not the spinal cord, GABAergic neurons promoted recovery in behavioral tests. MGE, medial ganglionic eminence.

somatic cells into induced pluripotent cells (iPSCs) that can subsequently be differentiated into neurons, or directly into induced neurons (iN cells), has entered two new players into the field, capable of providing patient- and disease-specific neurons on demand (Chambers and Studer, 2011). In this new arena, ESCs are up for a tough competition. While iPSCs suffer from the same safety concerns as ESCs, the ethical issues are less problematic and the ability to make donor-matched neurons offers a distinct advantage. Human iN cells obtained by direct conversion of somatic cells are the youngest players in the field and have yet to prove survival and efficacy in animal models of neurodegenerative disease. Nevertheless, the fact that these cells do not pass through a pluripotent state reduces the risk of uncontrolled proliferation and speeds up the conversion process.

The recent advancements in hESC differentiation and specification suggest that when the neuralization of hESCs is efficient and synchronized and recapitulates developmental signals for regionalization and subtype-specific differentiation, the resulting neurons are authentic, survive well, and do not form tumors in the host brain after transplantation, taking this strategy one step closer to clinical translation. However, more roadblocks have to be passed before hESCs are ready for clinical use. The use of ESCs as starting material is fraught with ethical issues, in addition to safety concerns associated with their pluripotent nature. Cell potency assays directly comparing the restorative potential of hESC-derived neurons to that of fetal neurons known to function in a clinical setting also need to be performed before progressing to the clinic. Furthermore, the absence of unwanted proliferation after grafting has to be much more rigorously tested in different models (including primates) and over longer time frames before clinical use can be considered (Lindvall et al., 2012). Despite these hurdles, the current advancements in the generation of fully functional striatal projection neurons from hESCs are an important step forward.

## REFERENCES

Aubry, L., Bugi, A., Lefort, N., Rousseau, F., Peschanski, M., and Perrier, A.L. (2008). Proc. Natl. Acad. Sci. USA *105*, 16707–16712.

Chambers, S.M., and Studer, L. (2011). Cell *145*, 827–830.

Chambers, S.M., Fasano, C.A., Papapetrou, E.P., Tomishima, M., Sadelain, M., and Studer, L. (2009). Nat. Biotechnol. *27*, 275–280.

Gögel, S., Gubernator, M., and Minger, S.L. (2011). Gene Ther. *18*, 1–6.

Kriks, S., Shim, J.W., Piao, J., Ganat, Y.M., Wakeman, D.R., Xie, Z., Carrillo-Reid, L., Auyeung, G., Antonacci, C., Buch, A., et al. (2011). Nature *480*, 547–551.

Lindvall, O., Barker, R.A., Brüstle, O., Isacson, O., and Svendsen, C.N. (2012). Cell Stem Cell *10*, 151–155.

Ma, L., Baoyang, H., Liu, Y., Vermilyea, S.C., Liu, H., Gao, L., Sun, Y., Zhang, X., and Zhang, S.C. (2012). Cell Stem Cell *10*, this issue, 455–464.

Pankratz, M.T., Li, X.J., Lavaute, T.M., Lyons, E.A., Chen, X., and Zhang, S.C. (2007). Stem Cells *25*, 1511–1520.

Roy, N.S., Cleren, C., Singh, S.K., Yang, L., Beal, M.F., and Goldman, S.A. (2006). Nat. Med. *12*, 1259–1268.

Thomson, J.A., Itskovits-Eldor, J., Shapiro, S.S., Waknitz, M.A., Swiergiel, J.J., Marshall, V.S., and Jones, J.M. (1998). Science *282*, 6467–6477.