Development of human neural stem cell lines for the treatment of Parkinson's disease

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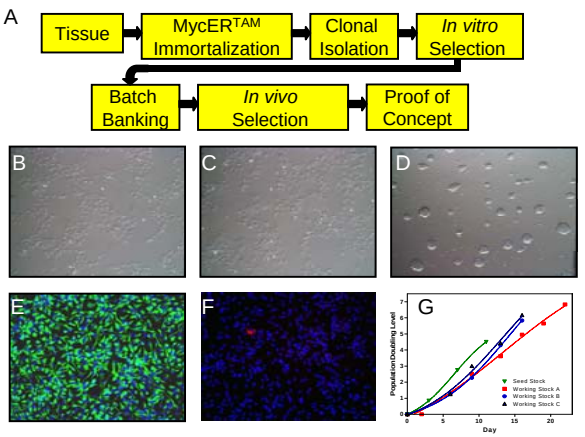
Introduction

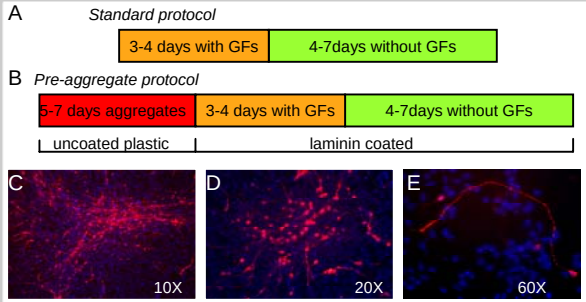
Our goal is to select clonal human neural stem cell lines with the potential to treat Parkinson's disease. Neural stem cells, obtained under US national ethical and legal guidelines, from the mid-brain region of first trimester fetal tissue from two donors, were expanded in culture and conditionally immortalized using our proprietary c-MycER^{TAM} technology. Clones were screened for their *in vitro* ability to differentiate into tyrosine hydroxylase-expressing neurons (TH, a marker for dopaminergic phenotype). From 21 clones, four karyotypically normal clonal TH-expressing cell lines emerged. These were also tested for their ability to survive *in vivo* following transplantation into normal rat brains.

Stem cell line derivation



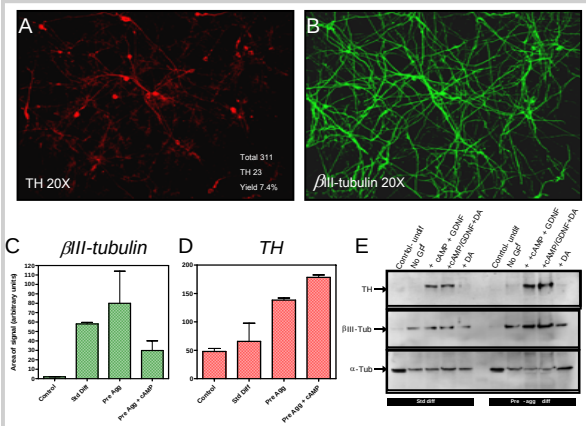
A) Process map defining stages in the development of new cell lines. B,C) Images of two undifferentiated monolayer clonal cell lines derived from two different donors. D) In the absence of laminin the clonal cell lines form discrete neurosphere bodies. All cells are nestin positive (E, green) and show a low level of TH differentiation in the proliferating culture (F, red) with Hoechst (blue). G) Growth curves of several batches made for one cell line show consistent growth rates.

Differentiation strategies



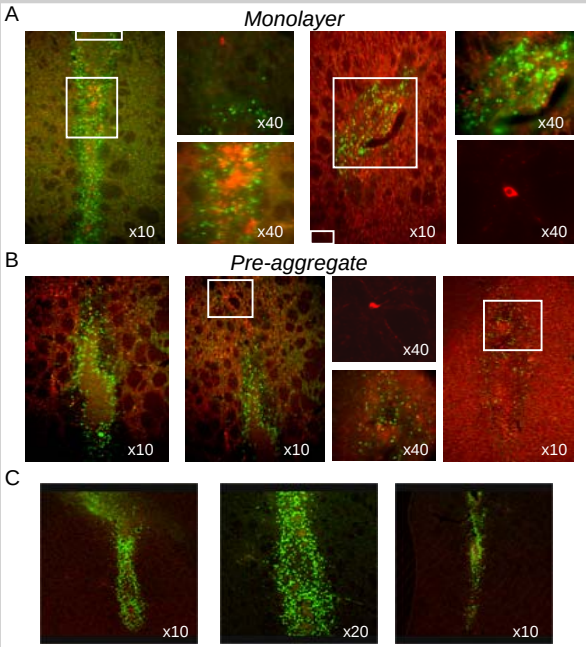
A,B) Schematic of two different differentiation paradigms used to screen *in vitro*. C-E) MycER transduced bulk mid-brain population, from which clonal cell lines were derived, shows robust TH (red) differentiation using the standard protocol with Hoechst nuclear stain (blue).

Dopaminergic differentiation



A,B) Images of *in vitro* differentiated mid-brain cell line. βIII-tubulin is shown in green and TH in red. C,D) Image analysis reveals that cAMP/GDNF enriches TH+ve neurons. E) Western blot analysis of the same cell line using dopamine (DA) pre-treatment or cAMP/GDNF to induce differentiation.

In vivo transplantation



A) Monolayer undifferentiated and B) pre-aggregate preparations of the same clone transplanted into the ipsilateral striatum of a unilateral substantia nigra sham lesion rat. Good survival was realized with all clones using both preparation methods at 1 month. Host TH+ve cell bodies were observed in the striatum with two of the clones. C) Five day survival of a monolayer preparation transplanted in the striatum of a 6-OHDA lesion rat model. Human nuclear antigen in green and TH shown in red.

Conclusion

We conclude that our selected lines have attributes that may favor the reversal of neurological deficits seen in Parkinson's disease. Transplantation studies are ongoing to assess the potential of these cell lines in a rodent model of Parkinson's disease.