

Recombinant Proteins to Induce Pluripotent Stem Cells: Promises for a Safer and Thriving Step Toward Clinical Trials

Rhee YH, Ko JY, Chang MY, et al. Protein-based human iPSCs efficiently generate functional dopamine neurons and can treat a rat model of Parkinson disease. *J Clin Invest* 2011;121:2326–2335.

Embryonic stem cells (ESCs) offer an unlimited source of replacement material for regenerative medicine to potentially treat many disabling diseases, including PD, or other neurodegenerative conditions. However, using ESCs for cellular therapy raises a number of ethical and medical problems, as their production requires the destruction of human embryos and allograft necessitates immunosuppressive treatment. To overcome these problems, the recent technology of induced pluripotent stem cells (iPSCs) allows the reprogramming of adult somatic cells into genuine pluripotent stem cells by introducing four main genes (*Oct4*, *Sox2*, *Klf4*, and *cMyc*) into their genome.¹ iPSCs have become a tool for the development of cellular therapy techniques because they permit autologous transplantation, thus obviating problems associated with immunosuppressive treatment.² Although holding great promise for personalized regenerative medicine, one crucial aspect is the genetic integrity of iPSCs. Indeed, viral vectors were used, at first, to introduce reprogramming genes that are irreversibly integrated and may induce uncontrolled proliferation of transplanted cells until tumor formation. Over 3 years, iPSCs research has expanded exponentially, and new virus-free methods have been developed to induce pluripotency (e.g., excising introduced exogenous genes to remove vector traces from iPSCs genome³ or using nonintegrative recombinant proteins⁴). Recently, Rhee et al. published an elegant study (*J Clin Invest* DOI: 10.1172/JCI45794) comparing differentiation and cellular properties of lentivirus-, retrovirus-, and protein-based human iPSCs to human ESCs. Optimized coculture and fine selection methods lead to the efficient generation of neural precursor cells (NPCs) and dopaminergic (DA) neurons from all iPSC lines; however, virus-based iPSCs presented limited expansion and early senescence, compared to protein-based and ESCs. Moreover, residual expression of exogenous genes was observed

in virus-based, but not in protein-based, iPSCs. In the face of these results, the investigators selected the safer line for therapeutic in vivo application in a rodent model of PD. NPC grafts into the striatum resulted in striking behavioral recovery associated with a high proportion of TH⁺ neurons, although tumor generation was observed when a high concentration of NPCs was grafted. Importantly, the results also confirmed that fully differentiated DA neurons are too vulnerable to survive transplantation, as no functional recovery and no TH⁺ neurons were observed in this case. This study elegantly showed the superiority of virus-free iPSCs, although further optimization is still needed to remove residual undifferentiated cells and associated tumor risk. Such work should pave the way for standardized quality control before moving iPSCs into clinical trials.

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