Lentiviral Gene Transfer to iPS Cells: Toward the Cardiomyocyte Differentiation of Pompe Disease-Specific iPS Cells

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iPS cells • Lentivirus • Pompe disease

Pompe disease is an inherited neuromuscular disorder caused by a genetic deficiency of acid-glucosidase-alpha (GAA). The clinical symptoms of Pompe disease include progressive weakness, respiratory failure, and ventricular hypertrophy. Enzyme replacement therapy has been shown to ameliorate these symptoms. Cardiomyocytes derived from patient/disease-specific iPS cells (iPS-CMs) have been used for pathophysiological analyses, drug screening, and cell therapy. Our research goal was to generate cardiomyocytes that can be differentiated from gene-corrected Pompe disease-specific iPS cells.

We obtained iPSC (TkDA3-4) generated from human dermal fibroblasts [1]. GAA was cloned into cDNA expressing third-generation lentiviral vectors (CS2-EF1α-GAA). To assess the transfection efficacy, Venus, a YFP variant protein, was also cloned into the vector (CS2-EF1α-Venus). Then, we transfected lentiviral vectors containing GAA to iPSCs at three different concentrations to

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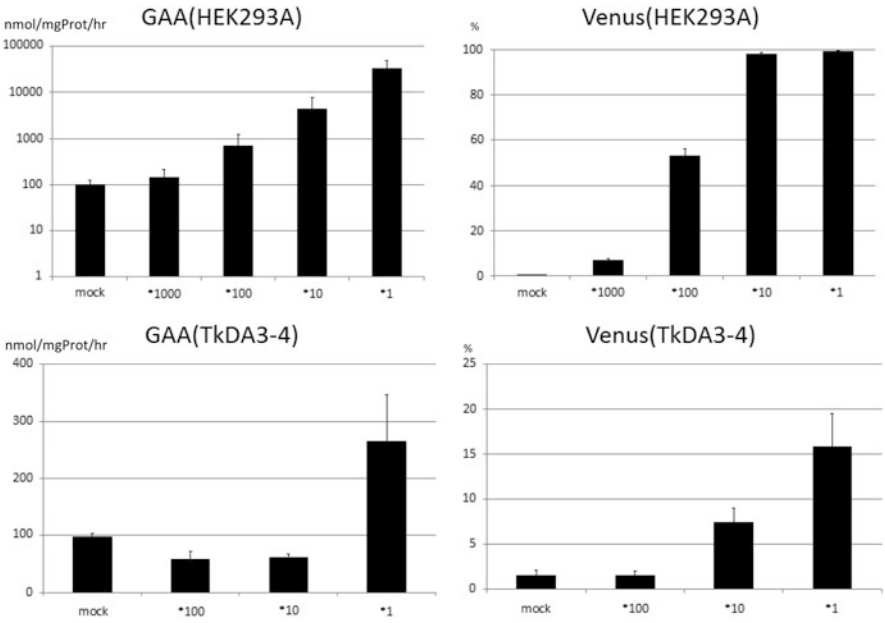

Fig. 48.1 Gene transfer to HEK293A cells and TkDA3-4. Dose-dependent expressions of GAA and Venus were observed in each cell line

determine the optimized titer for gene correction. We showed that dose-dependent expression of both GAA and Venus was observed in iPSCs, even though the expression levels were relatively low compared to HEK293A cells.

Cardiomyocyte differentiation of iPS cells is the most important procedure for replicating the disease hallmarks of Pompe disease. In fact, there is no single best protocol for obtaining cardiomyocytes derived from iPS cells. The functional assessment of iPSC-derived cardiomyocytes is another critical aspect of our research. The differences between the function of iPSC-derived cardiomyocytes obtained from normal control cells and those obtained from Pompe disease cells should therefore be strictly evaluated in order to thoroughly discuss the efficacy of gene therapy for iPSC (Fig. 48.1).

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Reference

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