

High-efficiency RNA-based reprogramming of human EB somatic cells

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No effective treatments are available for epidermolysis bullosa (EB), a group of rare inherited blistering disorders that can be devastating and in some cases lethal. The technological breakthrough that allows adult skin cells to be reprogrammed into induced pluripotent stem cells (iPSC) now offers the possibility of developing a permanent corrective therapy for EB. In a clinical scenario, skin cells can be biopsied from a patient suffering from EB and then “reprogrammed” into iPSCs. The iPSCs can then be grown outside the body, genetically corrected, differentiated into new skin stem cells, and administered back to the same patient as an autograft. The generation of iPSCs from the same patient in need of treatment would not only potentially avoid the complication of immune rejection, but also provide an unlimited and scalable source of patient-specific cells suitable for transplantation. Despite the almost limitless therapeutic potential of iPSCs for tissue repair, several obstacles must still be overcome before iPSCs can be applied in the clinic. One of these obstacles is the low efficiency and inconsistency of clinically relevant, integration-free approaches for the reprogramming of patient’s somatic cells into iPSCs. In the current presentation, we show that the reprogramming of patient’s fibroblasts into iPSCs can be significantly enhanced via the synergistic activity of reprogramming modified mRNAs and mature miRNA mimics in combination with optimized culturing conditions. When human primary neonatal fibroblasts are used, we can achieve an unprecedented reprogramming efficiency of ~ 800% (up to 4,019 TRA-1-60 positive colonies from 500 input fibroblasts) and can reprogram up to 90.7% of individually plated cells. Although the efficiency of our protocol drops when applied to adult fibroblasts, it remains extremely high, reaching 70% for cells isolated from healthy individuals. Not only does our protocol consistently generate iPSCs with extremely high efficiency from healthy individuals, but also from adult patients with a variety of disorders, including inherited skin diseases. For example, using this methodology, we have successfully generated iPSCs from fibroblasts isolated from patients with EB simplex and recessive dystrophic EB (RDEB) with the efficiency reaching ~ 25%. All iPSC lines exhibited normal karyotypes and have been successfully maintained for at least 15 passages. The pluripotency of the generated iPSCs was confirmed by gene expression analysis and the differentiation into cell types of all three germ layers both *in vitro* and *in vivo*.

To validate our protocol on a different cell type, we started exploring urine samples as an alternative non-invasive source of somatic cells for reprogramming. As a result, we can now successfully establish renal epithelial cell lines from just 30 ml of urine. We also optimized our reprogramming protocol and can now successfully reprogram renal epithelial lines with the RNA-based approach.

Our advances in developing safer cell reprogramming allowed us to enter into a Consortium with Stanford (Drs. Anthony Oro and Marius Wernig) and Columbia (Dr. Angela Christiano) Universities to develop an iPSC-based gene therapy for RDEB and advance this therapy toward a Phase I Clinical Trial. The EB Research Partnership, the EB Medical Research Foundation and the SOHANA Research Fund currently support the “EB iPS Cell Consortium”. The EB Consortium has already validated our high-efficiency RNA-based reprogramming technology as the method of choice for generating patient-specific iPSCs. We have recently combined our RNA-based reprogramming with gene correction into a one-step procedure, which will allow us to simplify the manufacturing of *COL7A1*-corrected autologous iPSCs for potential clinical applications.