Treatment of Recessive Dystrophic Epidermolysis Bullosa by Transplantation of *COL7A1* Transgenic Tissue-engineered Skin Grafts

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Abstract

Recessive dystrophic epidermolysis bullosa (RDEB), caused by loss of function mutations in the *COL7A1* gene encoding type VII collagen, is a devastating, often fatal, inherited blistering disorder lacking curative therapies. Epidermal stem cells/keratinocytes derived from skin tissue can be expanded, engineered with functional *COL7A1* gene and further differentiated into the whole epidermis in vitro, which hold great promise for the treatment of RDEB. Here, we generated a gene-corrected tissue-engineered skin graft from RDEB epidermal stem cells via non-viral vector-Piggy/Bac transposon under the serum-free and chemical-defined culture media. The resultant COL7A1 transgenic tissue-engineered skin graft could integrate into mouse skin for a long period, restoring the COL7A1 expression without tumorigenicity risk. Moreover, clinical data from Investigator-Initiated trial (IIT) showed good efficacy and tolerance on RDEB patient indicated with partial restoration of the COL7A1 expression, good wound healing and no adverse effects after autologous *COL7A1* transgenic tissue-engineered skin graft transplantation. Collectively, our *COL7A1* transgenic tissue-engineered skin grafts are promising for RDEB treatment in the future clinical study.

Figure 1. Restoration of COL7A1 expression in RDEB epidermal stem cells

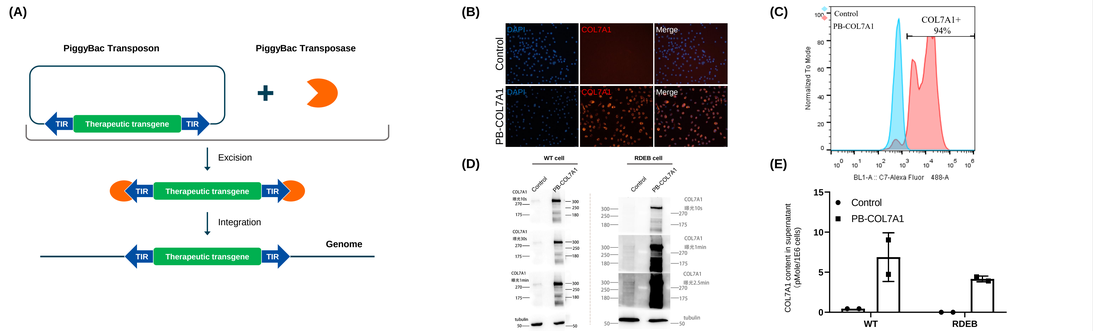
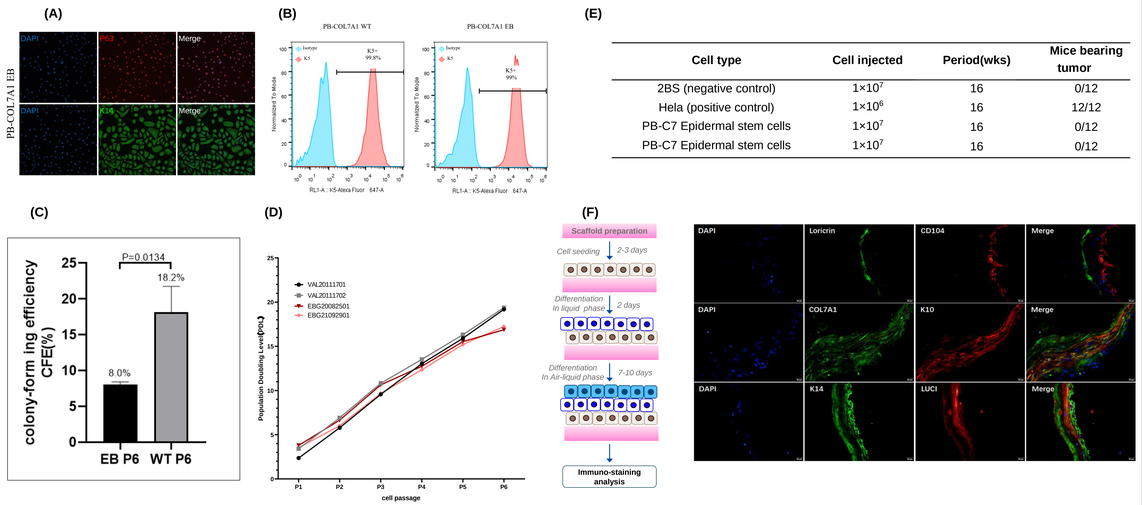


Fig 1. Piggy/Bac transposon-mediated the integration of a full-length human COL7A1 cDNA efficiently restored the COL7A1 expression in RDEB epidermal stem cells. (A) Working model of Piggy/Bac transposon system. (B) & (C) IF staining and flow cytometry analysis of COL7A1 showed efficient correction mediated by Piggy/Bac transposon system. (D)&(E) Western blot and ELISA assay targeting COL7A1 indicated a significant increase of COL7A1 level in in COL7A1-corrected RDEB epidermal stem cells.

Figure 2. Characterization of *COL7A1* transgenic epidermal stem cells



(E)

Cell type	Cell injected	Period(wks)	Mice bearing tumor
2BS (negative control)	1×10^7	16	0/12
Hela (positive control)	1×10^6	16	12/12
PB-C7 Epidermal stem cells	1×10^7	16	0/12
PB-C7 Epidermal stem cells	1×10^7	16	0/12

Fig 2. *COL7A1*-corrected RDEB epidermal stem cells maintained the characteristics of epidermal stem cells and were not tumorigenic. (A)&(B) IF staining and flow cytometry analysis of *COL7A1*-corrected RDEB epidermal stem cells indicated their uniform expression of markers like P63, K14 and K5 in basal layer of epidermis. (C)&(D) *COL7A1*-corrected RDEB epidermal stem cells could expand well in vitro indicated by proliferation and colony-forming assay. (E) *COL7A1*-corrected RDEB epidermal stem cells were not tumorigenic. (F) *COL7A1*-corrected RDEB epidermal stem cells could differentiate into the whole epidermis consisting of *Stratum basale* (K14+CD104+), *Stratum spinosum*(K10+), *Stratum granulosum*(Loricrin) and *Stratum corneum* in vitro.

Figure 3. Long-term integration and COL7A1 expression of *COL7A1*-corrected epidermal stem cell-derived tissued-engineered skin grafts in vivo

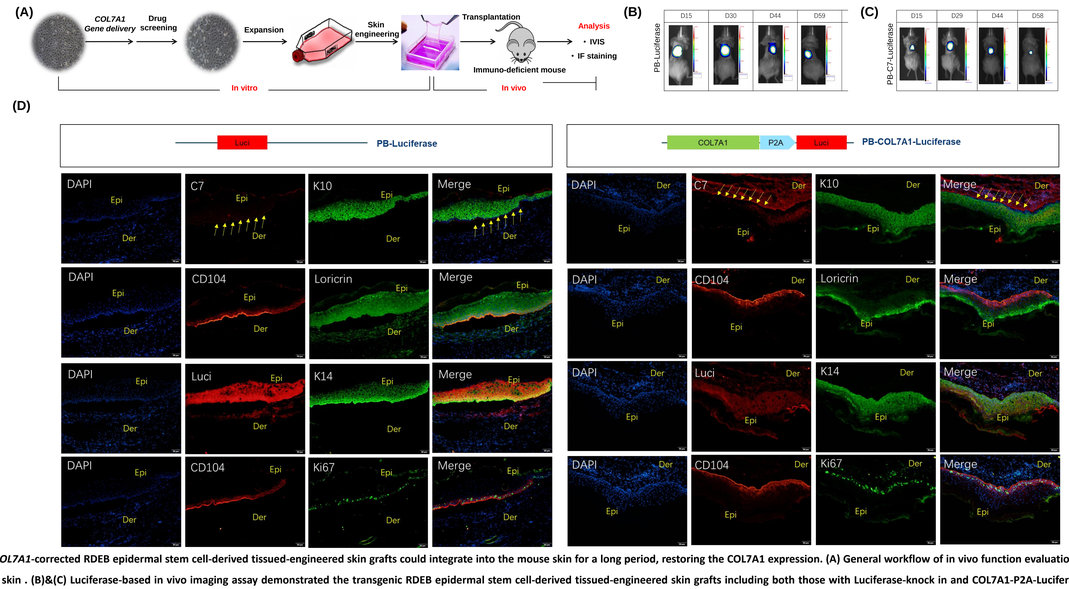


Fig 3. The *COL7A1*-corrected RDEB epidermal stem cell-derived tissued-engineered skin grafts could integrate into the mouse skin for a long period, restoring the COL7A1 expression. (A) General workflow of in vivo function evaluation of tissued-engineered skin . (B)&(C) Luciferase-based in vivo imaging assay demonstrated the transgenic RDEB epidermal stem cell-derived tissued-engineered skin grafts including both those with Luciferase-knock in and COL7A1-P2A-Luciferase-knock in could integrate and survived for a long period in vivo. (D) IF staining of the sections from the grafted area showed that the transgenic RDEB epidermal stem cell-derived tissued-engineered skin grafts could further differentiate into the whole epidermis consisting of *Stratum basale* (K14+CD104+), *Stratum spinosum*(K10+), *Stratum granulosum*(Loricrin) and *Stratum corneum*. Meanwhile, cells in the *Stratum basale expressed Ki67,* suggesting the self-renewal capacity. *COL7A1*-corrected RDEB epidermal stem cell-derived tissued-engineered skin grafts showed restored COL7A1 expression that effectively prevented the detachment of epidermis.

Figure 4. Autologous COL7A1-corrected tissued-engineered skin graft transplantation on RDEB patient

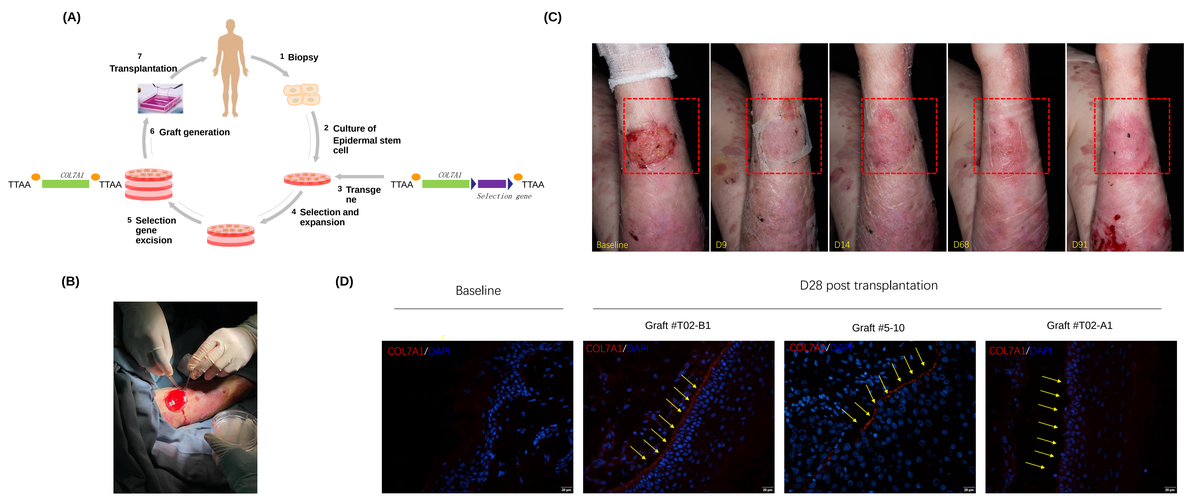


Fig 4. Autologous transplantation of *COL7A1*-corrected tissue-engineered skin grafts on RDEB patient showed good efficacy and tolerance. (A) General design of the treatment of RDEB by autologous transplantation of *COL7A1*-corrected tissue-engineered skin grafts. (B) in transplantation surgery. (C)&(D) good wound healing and partial restoration of the COL7A1 expression were observed after autologous COL7A1 transgenic tissue-engineered skin graft transplantation.

Conclusions

- *COL7A1*-corrected tissue-engineered skin grafts could be generated from RDEB epidermal stem cells via non-viral vector-Piggy/Bac transposon under the serum-free and chemical-defined culture media.
- *COL7A1*-corrected tissue-engineered skin grafts could integrate into mouse skin for a long period, restoring the COL7A1 expression without tumorigenicity risk.
- Our *COL7A1*-corrected tissue-engineered skin grafts are promising for RDEB treatment in the future clinical study.