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SHORT COMMUNICATION

Long-term Follow-up of Cultured Epidermal Autograft in a Patient with Recessive Dystrophic Epidermolysis Bullosa

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Recessive dystrophic epidermolysis bullosa (RDEB; MIM 226600) is characterized by mechanical stress-induced blistering of the skin and mucous membranes, followed by scarring and nail dystrophy (1). RDEB is caused by mutations in the *COL7A1* gene encoding type VII collagen (COL7), the major component of anchoring fibrils beneath the lamina densa (1, 2). No definitive treatments have been established (3). Chronic skin ulcers in patients with RDEB occasionally lead to aggressive squamous cell carcinoma (SCC), which often metastasizes and leads to death (4). Various kinds of biological dressings, including cultured autologous or allogeneic epidermal and/or dermal grafts, have been used to treat intractable ulcers (5). Cultured epidermal autograft (CEA) has been actively used in the treatment of ulcers, such as burns and skin graft donor sites, because it does not provoke immune rejection. In RDEB, there are a few successful reports of CEA treatment (6), but no information on long-term follow-up of the patients.

CASE REPORT

The patient was a 12-year-old Japanese boy who had developed generalized blisters induced by minor trauma since birth (Fig. 1a). Immunofluorescence showed a slightly reduced expression of COL7 at the dermal-epidermal junction, and transmission electron microscopy showed anchoring fibrils to be thin, poorly formed and reduced in number (7). Mutation analysis of genomic DNA demonstrated compound heterozygous mutations for G2576R and E2857X in the *COL7A1* gene (4, 7). These findings led us to diagnose him as RDEB, generalized other.

The CEA was manufactured by Japan Tissue Engineering Co. Ltd, using the Green method, as described previously, with some modifications (8, 9). A full-thickness biopsy specimen of skin (0.35 cm^2) was taken from the dorsum, which had not been involved. Keratinocytes were subcultured once or twice, and cryopreserved at –150ºC until transplantation. To prepare grafts, keratinocytes were thawed and cultured on a feeder layer to confluence, followed by detachment of cell sheets from flasks with dispase.

After informed consent was obtained from the patient and his parents, the patient received the CEA. The surface of the designated skin ulcers (the right shoulder, axilla, knee (Fig. 1a) and abdomen) was sterilized with chlorhexidine gluconate and saline solution. The CEA was applied to the wound surface, together with non-adherent siliconized gauze and bacitracin and fradiomycin ointment. The CEA was fixed with bandages.

At 3 days after the CEA procedure, spotted epithelialization was observed on the right knee (Fig. 1b), and at 2 weeks, almost full re-epithelialization was observed (Fig. 1c). However, the engraftments failed on the right shoulder, axilla and abdomen because of mechanical displacement, and these ulcers

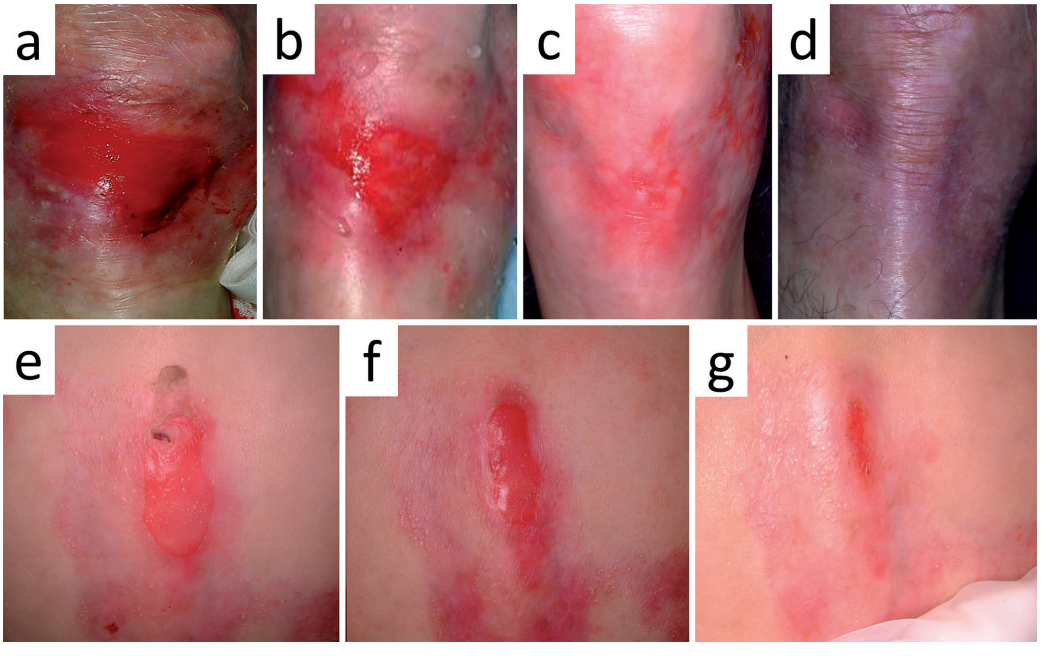

Fig. 1. (a) Intractable ulcer on the right knee. (b) Three days after cultured epidermal autograft, spotted epithelialization is observed, and (c) at 2 weeks, almost all full-epithelialization is observed. (d) Ten years later, a scar is observed at the successfully grafted area. The flexibility and texture of the lesion is similar to other scars that have re-epithelialized spontaneously. (e) Control ulcer. At approximately the same time, an ulcer was produced on the back and treated with ointment application. The lesion re-epithelialized from the border of the wound in a slow manner. The lesion at (f) 3 days and (g) 2 weeks.

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followed a protracted course before full re-epithelialization. Around the same time, a tractable ulcer was produced on the back and treated with ointment application (Fig. 1e). The lesion re-epithelialized from the border of the wound in a slow manner (Fig. 1f, g). Ten years later, scarring was observed at the successfully grafted area, and the flexibility and texture of the successfully grafted lesion was similar to the scars that had been re-epithelialized spontaneously (Fig. 1d). Notably, lesions that underwent successful CEA transplant had fewer ulcer recurrences than lesions that did not undergo successful CEA transplant.

DISCUSSION

A serial subculture technique has enabled the preparation of epithelial sheets using human keratinocytes (8, 9). Since then, cultured autologous and allogeneic epithelia have been produced for the treatment of extensive full-thickness burns, with good results (10). In addition, these biological dressings have been used for patients with EB with some success (6). However, controversy remains as to whether the process of isolating and culturing keratinocytes prior to transplantation may somehow induce genetic modifications or enhance cell stem properties, potentially generating an increased risk of tumourigenesis after transplantation. In this study, we used patient-derived keratinocytes that had been subcultured fewer than 3 times, and we carefully evaluated the cell morphology prior to the operation in order to reduce the possibility of carcinogenesis. To the best of our knowledge, only one case of graft site malignancy in a patient treated with CEA for burn injuries has been reported and the possibility of malignant transformation caused by the repeated ulceration and/or the culturing process were pointed out in that report (10). However, there has been no information on long-term follow-up of patients with EB treated with biological dressings and no cases of skin cancer or occurrence of hyperproliferative lesions associated with the graft in the literature. This study demonstrated a case of RDEB successfully treated with CEA and the long-term follow-up. More than 10 years after the CEA procedure, there was no evidence of tumourigenesis. CEA may be a potential treatment modality in RDEB patients with multiple ulcers, as it requires little donor skin.

Interestingly, the frequency with which ulcers recurred was significantly reduced on the area successfully transplanted with CEA, and there are several explanations for this observation. Various cytokines and other factors produced from cultured keratinocytes may influence the levels of mutated COL7. Therefore, it is possible that by increasing the dosage of COL7, the efficiency of CEA can be improved, thus, reducing the frequency of ulcers. Conversely, it is possible that the CEA may include some revertant keratinocytes, since the skin sample was taken from unaffected, intact dorsal skin. Further examination of the long-term safety and mechanism of action of CEA is required.

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Conflicts of interest. Drs Shinkuma, Sawamura, Fujita and Shimizu are investigators for a planned Clinical Trial for cultured epidermal autografts manufactured by Japan Tissue Engineering Co. Ltd and receive financial support from the company.

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