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Mini Review

Regenerative medicine for severe congenital skin disorders: restoration of deficient skin component proteins by stem cell therapy

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Some congenital skin disorders lacking structure proteins in the basement membrane zone carry severe prognosis because of severe erosion and skin dysfunction on the whole body. So far, several therapeutic strategies have been emerging for such disorders: 1. gene therapies, 2. protein therapies and 3. cell therapies. Cell therapies have a potential to affect skin systemically, and stem cell transplantation is one of the most hopeful candidates for treating severe congenital skin disorders such as epidermolysis bullosa, from a perspective of transdifferentiation and re-programming of stem cells. We review here the recent strategies and progress of stem cell transplantation for epidermolysis bullosa.

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Introduction

The skin is the human body's largest organ and accounts for approximately 16% of an adult's body weight. Several critical roles owe to the skin, including moderation of body temperature, prevention from electrolyte loss and protection from physical stimuli. In order to resist mechanical stress, the skin has complicated structures connecting epidermis and dermis, called basement membrane zone (BMZ) or dermal-epidermal junction. The BMZ consists of more than 30 structure proteins to strengthen the adhesion (Fig. 1)[1], and one defect of these proteins by congenital abnormality or acquired autoimmunity cause skin fragility and blister formation immediately after mild mechanical stimuli. Blistering on the whole body extremely worsens the quality-of life and even causes death due to severe water loss and infections.

Epidermolysis bullosa

One important example on the importance of BMZ proteins is epidermolysis bullosa (EB). EB comprises a group of inherited disorders in which the patient's epidermis can exhibit skin fragility caused by genetic abnormalities of a BMZ protein[2]. From the location of causative BMZ protein, EB is classified roughly into 3 categories: EB simplex (EBS), junctional EB (JEB) and dystrophic EB (DEB). Worldwide approximately 50 EB cases arise per a million live births and 92% accounts for EBS which is caused by cytokeratin 5/14 mutation with autosomal dominant inheritance[2]. Clinical manifestations vary broadly, from occasional mild erosion on the extremities to severe ulcers on the whole body or even stillbirth in Herlitz JEB and EBS/JEB with pyloric atresia. In recessive DEB, the most frequently recognized subtype in Japan, defect of type VII collagen (COL7) causes recurrent, deep erosions and ulcers on the extremities which results in mitten deformities and squamous cell carcinoma.

Emerging novel strategies for EB treatment

Most prevalent treatments for EB patients are skin protective care, wound dressing agents and antibiotics against local infections. There have been no established and fundamental treatments because EB arises from gene mutations of keratinocytes and fibroblasts on the whole body. However, several novel strategies have been emerging for EB treatment recently: 1. gene therapies, 2. protein therapies and 3. cell therapies.

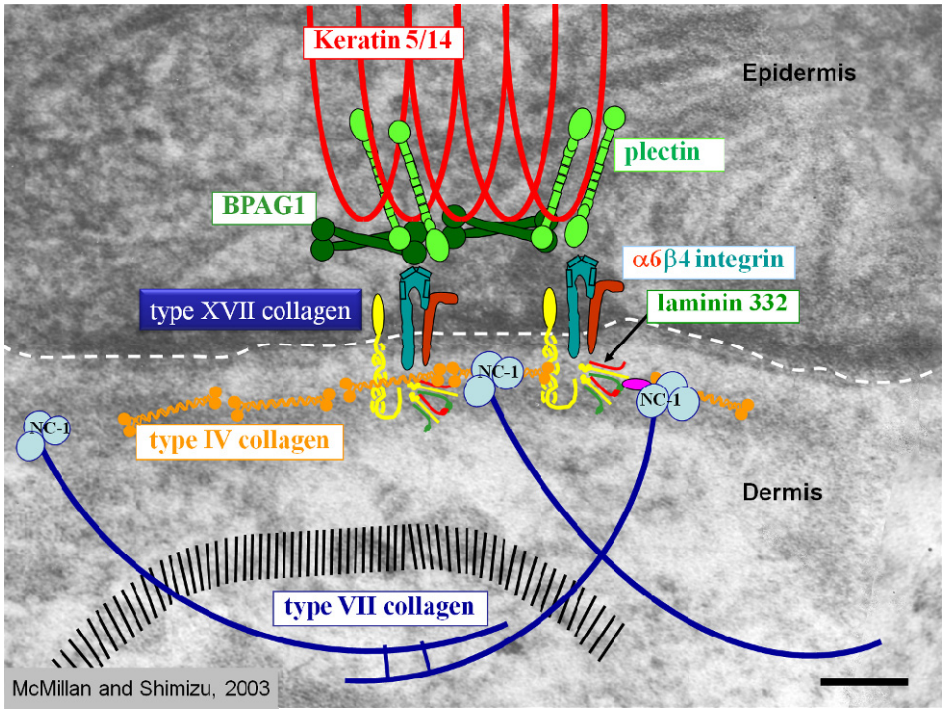

Fig. 1
Structure of basal membrane zone (BMZ) in the skin[1].

Gene therapies were performed by virus-mediated normal gene transfection into autologous keratinocytes, followed by cell culture to form epidermal sheet and grafting into the patients' skin. Such *ex vivo* gene-treated cultured autografting, reported by Mavilio *et al.*, is a promising therapeutic approach for junctional EB[3]. One of the merits of gene-mediated therapies is that autologous cells are fundamentally accepted without rejection response, except for the risk of immunoreactivity against the restored protein. Conversely, its effects are limited to the area of grafting and might be insufficient for systemic involvement of EB. Furthermore, the ethical and safety problems of using retroviruses for gene correction still exist[4]. Autologous induced pluripotent stem (iPS) cells are another source for gene therapies, since high proliferation potential provide enough number of differentiated cells without invasive techniques[5]. Successful treatment of sickle cell anemia model mice was recently reported by utilizing gene-corrected hematopoietic cell transplantation from autologous iPS cells[6]. Tolar *et al.* succeeded in the generation of autologous iPS cells from recessive DEB patient, which indicates that iPS-mediated therapies are theoretically possible by generation of epidermal/dermal sheets and hematopoietic stem cell transplantation[7]. However, ethical problems still lie on autologous iPS cells for the treatment of EB since gene correction by transfection is essential.

Conversely, few reports have been published as to *in vivo* gene therapies for EB[8]. As one candidate, several drugs have been reported to read through the specific stop codons of nonsense mutations, resulting in producing full-length proteins [9-11]. Therefore such "read-through" drugs might ameliorate severe congenital skin disorders if they are caused by the specific nonsense mutations. Since some subtypes of junctional EB have "hot spots" of nonsense mutations[12], there seems to be a space of novel gene-therapeutic agents in the future.

Congenital disorders that lack secretory proteins could be ameliorated by supplying the recombinant proteins systemically or locally. Several congenital metabolic disorders such as Fabry's disease have been already treated with enzyme replacement therapy[13]. Woodley and colleagues succeeded in the deposition of COL7 at the BMZ of artificially-constructed DEB skin by injecting recombinant COL7[14]. The same group later reported the amelioration of RDEB mice by injecting human COL7[15]. Other than secretory proteins like COL7, laminin beta-3, a structural protein in the BMZ, is found to be provided with protein therapy by protein transfection technique[16]. Protein therapies are safer than other novel therapies in the way that patients can attempt the therapy with lower dose of protein and that no gene correction is needed. Conversely, its effects are limited to the area of injection. The safety of the recombinant protein should be alarmed since bovine serum is generally essential for the culture of transfected cells. Efficient purification of large amount of protein is another challenge. The risk of immunoreactivity might weaken the effect of protein therapy and even cause exacerbation. In recessive DEB-generalized other type, the mutated COL7 protein partially function to form incomplete anchoring fibrils. Therefore, protein therapy-induced autoimmunity in such patients might inhibit the residual COL7 functions, resulting in exacerbation of blistering on the whole body.

Considering the clinical application of congenital disorders, the easiest source of normal proteins is allografts. Therefore, utilizing allogenic normal cells could be the fundamental therapeutic strategy. Applying allogenic keratinocytes, or allo-skin graft could treat congenital skin disorders, but allogenic keratinocytes are generally rejected because of their high immunogenicity. In order to overcome rejection, less immunogenic cells such as fibroblasts have been attempted to treat DEB. Intralesional injection of allogenic fibroblasts into DEB patients caused the deposition of COL7 for more than 3 months with matured anchoring fibrils[17]. Furthermore, intravenous injection of human fibroblasts into nude mice introduced human COL7 deposition in the BMZ of wound-healed skin[18]. Mesenchymal stem/stromal cells (MSCs) are another candidate for cell therapies; Conget and colleagues reported COL7 deposition at the site of intradermal injection of allogenic MSCs[19] in RDEB patient.

Another strategy of cell therapy is stem cell transplantation such as bone marrow transplantation (BMT) and cord-blood stem cell transplantation. If such stem cells engraft completely and provide functional stem cell-derived skin component cells from peripheral blood flow, systemic amelioration of EB will be accomplished for a long time without immunological rejection. Since stem cell transplantation has already performed widely for hematologic disorders and some congenital metabolic disorders, ethical and technical hurdles are much lower than gene/protein therapies.

Differentiation from bone marrow cells into functional keratinocytes

Stem cells in the bone marrow were recently found to have a pluripotency; a potential to differentiate into various cell lineages other than hematocytes. This pluripotency or transdifferentiation are observed more frequently in the injured organs such as damaged liver, ischemic heart, injured nerve tissues and wounded skin[20,21]. However, it had been unknown what causes efficient differentiation from bone marrow stem cells into injured skin, and whether these differentiated cells actually function like other normal organ cells.

Our group first revealed that a chemokine CTACK/CCL27 from the injured skin tissue accelerates the differentiation from bone marrow stem cells into epidermal keratinocytes[22]. Murine GFP-positive bone marrow cells were transplanted into normal mice, and the acceleration of wound healing and GFP-positive epidermal keratinocytes were investigated with or without local injection of CTACK/CCL27. Interestingly, CTACK/CCL27 enhanced the bone marrow-derived keratinocytes approximately 4 times, which was inhibited by anti-CTACK/CCL27 antibodies. Another chemokine SLC/CCL21 are similarly found to enhance wound healing via differentiating MSCs into various skin component cells including keratinocytes[23].

We also revealed that these differentiated keratinocytes actually function and provide BMZ component proteins. Focused on one basal keratinocyte-specific structural protein type XVII collagen (COL17), we prepared mice expressing normal murine Col17 (mCol17), transgenic mice expressing both murine and human COL17 (hCOL17) and COL17-humanized mice that express only hCOL17[24]. Interestingly, the expressions of donor bone marrow-derived COL17 in the skin were confirmed after performing BMTs among these mice of different COL17 expression patterns[25]. Since only keratinocytes express COL17 among skin-component cells and peripheral blood, bone marrow-derived keratinocytes are found to function and produce a BMZ component COL17.

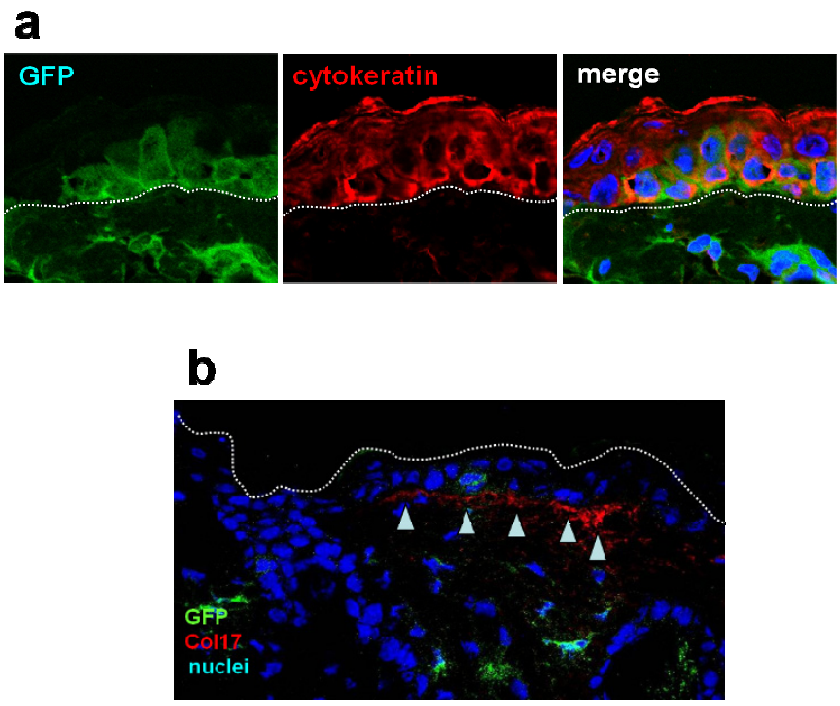

Fig. 2
Bone marrow transplantation into Col17 knockout JEB mice. (a) Donor-derived, GFP+ cytokeratin+ cells are aggregated in the basal cell layer of the epidermis, indicating bone marrow cells re-programmed into epidermal keratinocytes. (b) Immunofluorescence revealed GFP+ cells in the epidermis and dermis, with linear expression of Col17 in the BMZ.

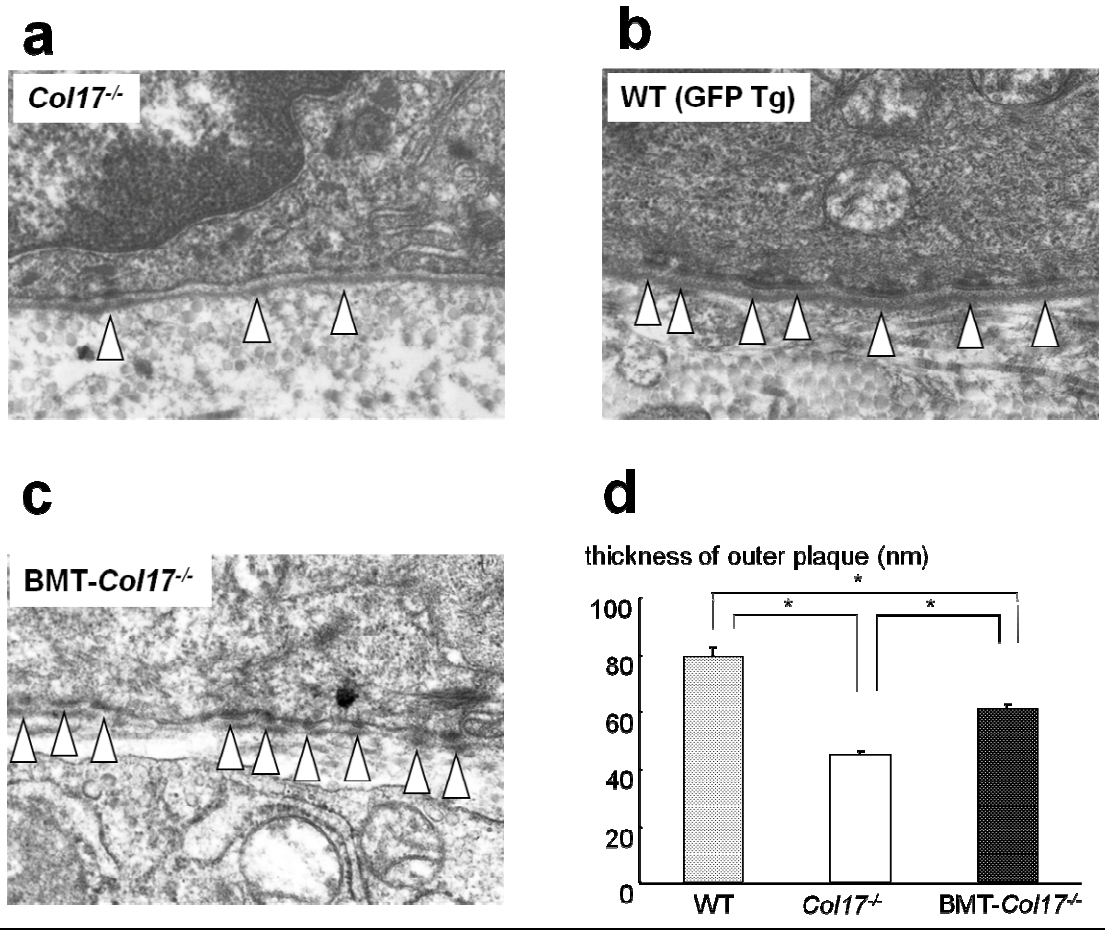

Fig. 3
Electron microscopy analysis in the skin after BMT into JEB mice. (a) Untreated Col17 knockout mice have thin, immature hemidesmosomes in the bottom of basal cell layer (arrowheads). (b) Normal C57BL/6 mice have mature, apparent hemidesmosomes. (c) Thick and matured hemidesmosomes are observed in the skin of BMT-treated Col17 knockout mice. (d) Thickness of the outer plaques of hemidesmosomes shows statistical improvement after BMT.

Stem cell therapy for epidermolysis bullosa

As mentioned previously, stem cell therapy is a promising strategy for systemic amelioration of EB for a long time. So far, a few investigations of BMT to treat RDEB have been published. Tolar *et al.* reported that hematopoietic stem cells contributed to life prolongation in RDEB model mice[26]. Chino *et al.* reported that treatment of embryonic BMT into RDEB model mice induced the expression of type VII collagen[27]. These reports proved the existence of donor-derived fibroblasts by immunohistochemistry and cell culture, and these fibroblasts are thought to produce type VII collagen. Based on these findings, hematopoietic stem cell therapies recently performed for RDEB patients in the US as a phase I/II clinical trial[28]. Five out of seven patients survived after the treatment, and less frequent dressings into the wound skin have achieved probably due to restoration of type VII collagen. These reports implied the benefit of stem cell transplantation in patients with deficient type VII collagen, which is produced by both epidermal keratinocytes and dermal fibroblasts[29]. Then, how is the clinical effect of stem cell transplantation in other subtype of EB, in which keratinocyte-specific skin component protein is lacked?

In order to answer the question we performed stem cell transplantation into adult Col17 knockout JEB model mice[25]. These treated mice expressed the lacked Col17 protein in the BMZ of the eroded skin around donor-derived GFP+ keratinocytes, with mature hemidesmosomes on the basal cells (Fig. 2, 3).

Clinical manifestations such as skin fragility and survival rates were also improved after stem cell transplantation (Fig. 4). Not only conventional BMT technique but hematopoietic stem cells transplantation and MSC infusion improved the expression of Col17. Furthermore, human hematopoietic stem cells also have a potential to restore epidermal component proteins by investigation of human-murine xeno-transplantation model, which implies stem cell transplantation might be a promising and fundamental therapeutic strategy for the treatment of severe EB patients.

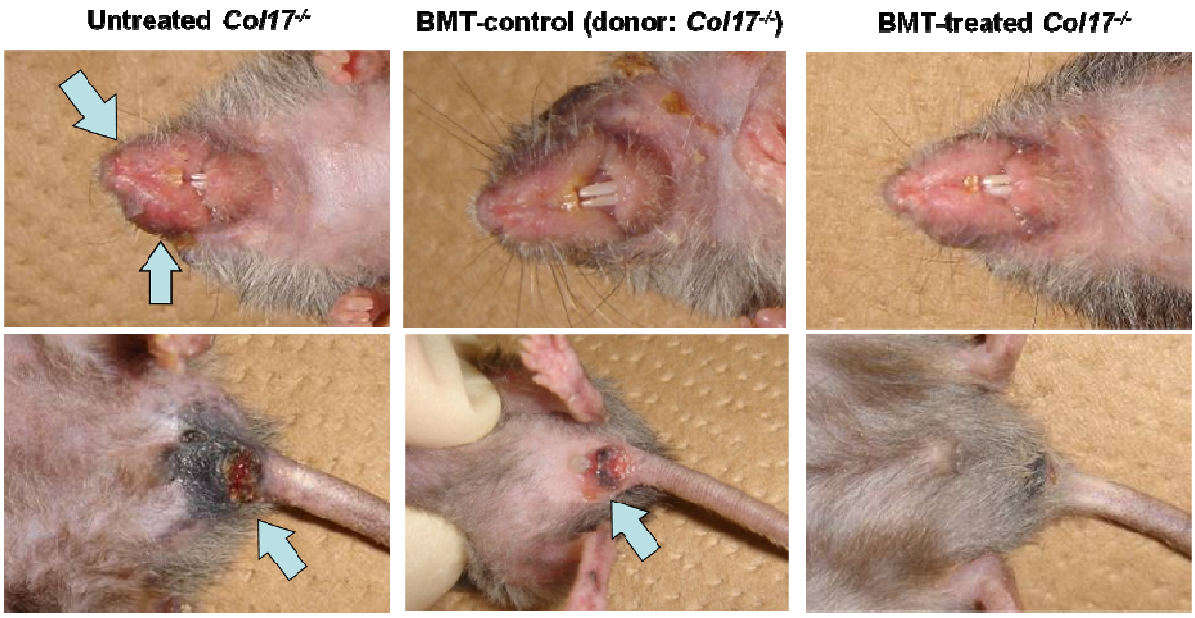

Fig. 4
Clinical manifestations of Col17 knockout mice after 120 days after birth (90 dayes after BMT). (left) Untreated Col17 knockout mice show erosions and ulcers on the perioral and perianal areas, which is compatible with clinical manifestations of JEB. (middle) As a control, BMT was performed from Col17 knockout mice into Col17 knockout mice. Severe erosions still appear as untreated mice. (right) Therapeutic BMT from GFP+ mice caused less severe erosions.

There still have problems to overcome on the stem cell transplantation for severe EB patients; *e.g.* risk of infection, conditioning regimens and donor supply. Although stem cell transplantation is prevalent, treatment-related deaths do occur due to severe infection, regimen-related toxicity and graft-versus-host disease (GVHD). Since EB patients have severe erosion and blisters on the whole body, severe cutaneous infections during the treatment could be fatal[28,30]. Conditioning regimens and the consideration of mini-transplantation should be determined carefully to avoid severe GVHD; both GVHD and regimen-related toxicity could cause severe erosions that are indistinguishable from EB symptoms. The donor is another challenge. Related HLA-matched siblings without EB phenotype are ideal for donors, but few cases meet the condition[28]. Unrelated HLA-matched stem cells from donor coordination programs, T-cell depleted haploidentical stem cell transplantation and iPS cell-bank projects might open the door to stem cell therapies in the future[31,32].

Concluding remarks

Stem cell therapies have been emerged as a promising strategy for congenital severe skin disorders such as EB. Although merits and demerits should be considered compared to gene therapies and protein therapies, novel treatments from the view of regenerative medicine will be one of the main streams to provide fundamental answers for severe disorders.

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