Adipose-Derived Stromal/Stem Cells for the Treatment of Skin Diseases

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Recessive dystrophic epidermolysis bullosa (RDEB) is the most severe form of epidermolysis bullosa, a group of genetic skin fragility disorders. Blisters, skin erosion, and scars form all over the body, including mucous membranes in patients with RDEB. The pathogenesis of RDEB involves mutations of the \( \text{COL7A1} \) gene encoding type VII collagen, the main constituent of anchoring fibrils that attach the epidermis to the dermis. Persistent skin erosion frequently results in intractable ulcers. As the ulcers heal, they result in severe scarring. Long-term inflammation of skin ulcers all over the body may cause secondary amyloidosis leading to chronic renal failure. In addition, patients with RDEB are at a high risk of skin cancer. Although there is no curative therapy for patients with RDEB, various kinds of biological dressings, including cultured skin substitutes, have been employed for the treatment of intractable ulcers. Nonetheless, allogeneic cultured skin cannot be permanently adopted. Autologous cultured skin lacks type VII collagen. Adipose-derived stromal/stem cells (ASCs) are easily harvested in large quantities from a minimal donor site, and show less immunogenicity and a powerful immunosuppressive potential. In addition, ASCs can differentiate into keratinocyte-like cells. Stem cell therapies using allogeneic ASCs may be applicable to the treatment of RDEB and other skin diseases in near future.

**Key words**: adipose-derived stromal/stem cell (ASCs), keratinocyte, fibroblast, collagen, epidermolysis bullosa

**Abbreviations**: EB; epidermolysis bullosa, RDEB; recessive dystrophic epidermolysis bullosa, Col7; type VII collagen, SCC; squamous cell carcinoma, ESC; embryonic stem cell, iPSC; induced pluripotent stem cell, MSC; mesenchymal stem cell, ASC; adipose-derived stromal/stem cell, DMEM; Dulbecco's Modified Eagle's Medium, ATRA; all-trans retinoic acid, BMP4; bone-morphogenetic protein-4, KSFM; keratinocyte serum-free medium, DSG3; desmoglein 3, K-5; cytokeratin-5.

**Recessive dystrophic epidermolysis bullosa**

Epidermolysis bullosa (EB) is a group of genetic skin fragility disorders, "mechanobullous diseases". Blisters and skin erosion form in response to minor injury or friction such as rubbing or scratching.

Recessive dystrophic epidermolysis bullosa (RDEB) is the most severe form of EB. Blisters, erosion, ulcers, and scars form all over the body, including mucous membranes (Figure-1). The pathogenesis of RDEB involves mutations of the \( \text{COL7A1} \) gene encoding type VII collagen (Col7), which is synthesized by both basal keratinocytes and dermal fibroblasts in human skin\(^1\)\(^,\)\(^2\). Col7 is the main constituent of anchoring fibrils that anchor the epidermal basement membrane to the papillary dermis\(^3\). Thus, patients with RDEB experience blistering and repeated wounding of the skin, oral mucosa, and gastrointestinal tract\(^4\)\(^,\)\(^5\).

In patients with RDEB, persistent skin erosion frequently results in intractable ulcers. The
symptoms are recalcitrant, and the ulcers are mostly painful. Wound pain reduces RDEB patients’ activities of daily living and quality of life significantly. As the ulcers heal, they result in severe scarring, fusions of fingers and toes (pseudosyndactyly), loss of nails, and joint contractures (Figure-2). Pseudosyndactyly is a common phenomenon in patients with RDEB and is surgically treated by releasing with scissors and full-thickness skin grafting. In nonsevere cases, fingers are separated using only a CO₂ laser without skin grafting several times (Figure-3). Scarring of the esophagus makes it difficult to swallow food, and is treated with balloon catheter dilatation. Long-term inflammation of skin ulcers all over the body causes secondary amyloidosis. Renal failure
due to secondary amyloidosis is an important cause of mortality\textsuperscript{8-10}. It was reported that an RDEB patient with chronic renal failure was treated with hemodialysis using a permanent vascular catheter\textsuperscript{11}.

Patients with RDEB are at a high risk of skin cancer (squamous cell carcinoma; SCC, in particular), which tends to be aggressive. It is difficult to treat RDEB patients with autologous skin grafting because Col7 in the graft is also mutated. Therefore, SCCs are usually excised without skin grafting (Figure-4). Life expectancy is significantly reduced due to frequent metastases.

**Treatment of RDEB**

So far there is no curative therapy for RDEB. Various kinds of biological dressings, including autologous and allogeneic cultured skin substitutes, have been used for the treatment of intractable ulcers in patients with RDEB (Figure-5)\textsuperscript{12-18}. Nonetheless, allogeneic cultured skin cannot be

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**Figure-3**

A. Pseudosyndactyly caused by scarring of the right hand in an adult patient with RDEB.
B. Fingers are separated by means of a CO\textsubscript{2} laser without skin grafting.

**Figure-4**

A. SCC of the right hand in an adult patient with severe generalized RDEB.
B. SCC was excised and covered with artificial dermis due to the difficulty of harvesting autologous skin.

**Figure-5**

An allogeneic cultured dermal substitute (made of a collagen sponge, hyaluronic acid, and fibroblasts) was placed on the intractable ulcer of the right hand in a patient with severe generalized RDEB.
permanently adopted. On the other hand, autologous cultured skin can serve as a permanent covering although its Col7 contains the mutations. Thus, both are used only as temporary biological dressings.

**Stem cells for the treatment of RDEB**

Regenerative medicine has begun to adopt novel treatments of various diseases. Among them, stem cells are a unique population of undifferentiated cells that have the ability to self-renew and differentiate into various cell types. These cells are expected to play a central role in the treatment of RDEB.

Stem cells are classified into embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), and adult stem cells. Ethical issues are current problems with the use of ESCs. On the other hand, preparation of iPSCs requires introduction of exogenous transcription factors; this process carries a risk of carcinogenesis. In contrast, adult stem cells circumvent ethical issues associated with ESCs.

Adult stem cells include hematopoietic stem cells, mesenchymal stem cells (MSCs), endothelial stem cells, and others. Among adult stem cells, MSCs are isolated from many kinds of adult tissues such as bone marrow, cord blood, peripheral blood, fetal lever and lungs, and adipose tissue. They have been shown to be capable of multilineage differentiation into osteocytes, adipocytes, myocytes, and chondrocytes. MSCs were also reported to promote wound healing. As a result, adult stem cells, including MSCs, alleviated RDEB both in an RDEB animal model and in human subjects.

**Adipose-derived stromal/stem cells**

Among MSCs, adipose-derived stromal/stem cells (ASCs) are easily harvested in large quantities from a minimal donor site (in contrast to bone marrow stromal cells) and have a differentiation potential similar to that of other MSCs. This means that ASCs are not only precursors of adipocytes but also multipotent progenitors for a variety of cell types. Moreover, ASCs show less immunogenicity and a powerful immunosuppressive potential. Accordingly, ASCs are suitable for not only an autologous but also allogeneic transplant. To determine the utility of ASCs in an allogeneic transplant from a healthy donor to repair basement membrane alteration in RDEB patients, the potential of ASCs’ differentiation into keratinocytes and expression of Col7 in ASCs must be studied, because patients with RDEB contain mutated Col7.

Subcutaneous adipose tissue can be obtained from disease-free donors under local anesthesia. The extracellular matrix of the excised tissue is digested with collagenase; the resulting cell suspension is filtered through a 40-µm nylon mesh and centrifuged. The resulting cells, consisting mostly of ASCs, are seeded in culture dishes. After 7 days of culture in Dulbecco’s Modified Eagle’s Medium (DMEM), cells that adhere to the dish become spindle-shaped or fibroblast-like and test positive for CD34, CD44, and CD90. These cells can differentiate into adipocytes.

**Differentiation of ASCs into keratinocyte-like cells**

ASCs were cultured in DMEM containing 10% of fetal bovine serum on type IV collagen, and co-cultured with normal human fibroblasts. All-trans retinoic acid (ATRA) was added, and the cells were cultured for 3 days, then bone morphogenetic protein 4 (BMP4) was added. After 4 days culture, the ATRA- and BMP4-containing medium was replaced with keratinocyte serum-free medium (KSFM) for 7 more days of culture. In addition, ASCs were cultured in the KSFM medium on type IV collagen without co-culturing with other cells for 14 more days.

These cells showed a characteristic polygonal cobblestone shape of epidermal keratinocytes. They showed high expression of keratinocyte markers desmoglein 3 (DSG3) and cytokeratin 5 (K-5) as determined by real-time PCR. By utilizing immunocytochemistry, real-time PCR, and western blotting, we confirmed that ASCs could differentiated into keratinocytes, and also expressed more Col7 than undifferentiated ASCs (data not shown).

**The therapeutic potential of ASCs for the treatment of skin diseases**

ASCs are an easily harvested form of MSCs. The advantages of using adipose-tissue-derived ASCs
include their abundance in donors and the ease of production by relatively noninvasive methods such as liposuction. In addition, ASCs can differentiate into keratinocyte-like cells. Stem cell therapies using allogeneic ASCs may be applicable to the treatment of RDEB although anti-Col7 antibodies may be relatively common among patients with RDEB. Allogeneic ASCs may not only enhance wound healing, but also correct the Col7 insufficiency, repair defective/reduced anchoring fibrils, and improve skin integrity in the patients. Although the method for ASC administration is still subject to debate, these cells may be useful in therapies for RDEB and other skin diseases in near future.

Conflict of Interest

The author has no conflicts of interest to declare.

References


