Epidermolysis Bullosa, Challenges and Novel Therapies

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Structured Abstract

Background: Epidermolysis bullosa (EB) is a congenital and life threatening disease and a genetic disorder of connective tissue with a prevalence of 3500 patients in the United States of America until 2016. There are four major congenital types of disease including simplex, junctional, dystrophic and a newer type, kindler-be syndrome.

Method: In order to obtain data for this review article we have searched databases and resources of PubMed and Scopus. The search keywords included Epidermolysis bullosa, EB, treatment, Gene therapy, hemi desmosome and Stem cell. First, we studied the titles and abstracts of articles, then we reviewed the full texts of more relevant studies and data extracted from them.

Results: EB patients are at increased risk of death from their disease consequences before the third decent of them life. Gene correction and stem cell transplantation or combination of tissue engineering and gene therapy are modern suggested treatments for EB. This review spends to etiologies and mutations resulting in different types of the disease and efforts conducted in order to modify mutations, especially those affecting hemi desmosome structures in epidermal keratinocytes in different types of EB.

Conclusion: In vitro gene therapy of keratinocyte stem cells and transplant them back to the skin as a novel suggested treatment option is expected to improve clinical features in EB patients. Application of viral vectors for gene therapy is restricted due to ethical issues and usage of non-viral vectors is more applicable for gene therapy in these patients. Authors conclude from previous researches that there is a proportional relation between numbers of each different subunits of hemi desmosome in its structure (disturbed in EB), which have been neglected and should be considered in future gene therapy researches.

Keywords: Epidermolysis bullosa, EB, Gene therapy, Hemi desmosome, Stem cell

Graphical Abstract: (Figure 1)
Text Organization

Introduction

Epidermolysis bullosa is a congenital and life-threatening disease and a genetic disorder of the connective tissue with a prevalence of 3500 patients in the United States of America until 2016 [1]. There are four major congenital types of the disease including simplex, junctional, dystrophic and a newer form, kindler-be syndrome. Epidermolysis bullosa (EB) patients are at increased risk of death from their disease consequences, before the age of 30 [2]. In spite of numerous researches conducted in order to find the certain treatment, until now there is just some few clinical therapeutic options including wound dressing, daily inspection of wounds and analgesic treatments [3,4].

EB classification history: [5].

Now a day, there are four major types of EB according to numerous clinical and basic science researches done during decades. Those types including:

1) Simplex epidermolysis bullosa (EBS)

EBS subtype accompanies with mechanical fragility and blisters occur on the epidermis. Previously, EBS was named as “epidermolytic” then EBS categorized into basal and suprabasal subgroups, according to the site of defect within the epidermis.

2) Junctional epidermolysis bullosa (JEB)

In JEB, blisters occur at the junction, known as lamina Lucida, of the skin basement membrane zone (BMZ).

3) Dystrophic epidermolysis bullosa (DEB)

In the past DEB was known as “dermolytic” EB in which blistering arises within the upper segment of dermis.

4) Kindler-be syndrome

In 2008, Kindler-be syndrome added to the EB classification. Kindler syndrome manifests uniquely among other subtypes of EB by photosensitivity and blistering that develops in multiple segments of skin within and/or under the basement membrane zone (BMZ).

Four main types of EB

1) Epidermolysis bullosa simplex

The first main type of EB that initially manifests in neonatal period by diffuse blisters all over the body; especially in upper and lower extremities [6]. This type of the disease is the most prevalent subtype in western countries with milder skin lesions in comparison with junctional and dystrophic types [7].

Epidermolysis bullosa simplex with muscular dystrophy is a variant of simplex type of EB

Manifests by diffuse skin blisters and muscular dystrophy in older ages. This type of disease usually accompanies by pyloric atresia or hypoplasia of enamel [8].

Junctional epidermolysis bullosa

The second main type of the disease which contains of blisters arise inside the lamina Lucida layer of basement membrane. Junctional EB classify into three categories based on the severity of the disease including benign, non-lethal and lethal types. Lethal junctional EB type manifests by wounds around mouth, eyes and nose which subsequently accompany by granulation tissue formation. Furthermore systemic involvements such as lesions on cornea, conjunctiva, tracheobronchial airways, mouth, pharynx and esophagus, rectum and mucosa of genitourinary system and also cough and respiratory disorders are present in this type of disease. These patients are at high risk of sepsis and death [9].

2) Dystrophic epidermolysis bullosa

The third main type of the disease manifests by blisters which remains atrophic scars after resolving. Milias (white papules, 1-4 mm in diameter) are the other complication due to lesions to hair follicles. Involvement of nails are also expected. Lesions on mucosal surfaces will result in some events including atresia and stenosis of esophagus, anal atresia, urethral atresia, phimosis and corneal scar. Malnutrition and growth retardation are the other complications [10]. These patients owe the higher risk of involvement by SCC later in them life [11]. Furthermore, the dermal fibrosis is another complication in these patients [12].

3) Kindler-be syndrome

Kindler syndrome is an autosomal recessive disease manifests by photosensitivity, blistering resulted from trauma, skin atrophy and poikiloderma. Especially in early stages of this syndrome, clinical manifestations overlap with dystrophic type of epidermolysis bullosa, however currently kindler-be syndrome consider as a distinct type of epidermolysis bullosa [13].

In kindler syndrome, defects and reduplication in basement membrane zone have been shown by immunohistochemical and ultrastructural studies, leading to reduced cell proliferation, adhesion, spreading and undirected migration (multiple polarities cells). FFH1 (the main disrupted agent in kindler-be syndrome) establishes some interactions between ECM and membranous agents (beta 1 and beta 3 proteins) at focal adhesions [14]. There is also evidences show the increased risk of cutaneous and laryngeal squamous cell carcinoma in patients with kindler syndrome [15].

3 Diagnostic approach

The diagnosis of different types of the disease performs by firstly, evaluation of clinical features of patients and then by evaluation of mutations by means of molecular genetic testing. If the diagnosis didn’t establish by molecular genetic testing, histologic evaluation of skin biopsy by means of direct immunofluorescence (IF) or immunohistochemistry (IHC) will perform in order to determine defects in specific cellular components. Molecular studies are the only accurate and accepted method in order to diagnose and determine the type of disease [16-21].

4 Current treatments

Unfortunately, until now the treatment options for patients with EB is limited to the wound care and symptom relieve strategies and also some nonspecific immunosuppressive treatments in order to compensate the complications [17]. Numerous previous studies have focused on finding mutations of genes responsible for occurrence of the disease resulted in comprehensive classification of different types of EB according to
their clinical manifestations and suggested involved genes. One of the other therapies suggested is the implication of cultured stem cells to compensate the defect of keratinocytes in the skin wounds; as well as using skin grafts for long term closure of chronic wounds. In major of researches in the field of gene therapy for treatment of EB, implication of retroviral vectors have kept those studies on hold [22]. So the usage of viral vectors is the blind spot of those researches and an obstacle in the way of their clinical application.

Until now, despite conducted efforts for medication therapy in EB, no drugs or certain therapies approved. Phenytoin or tetracycline drugs which act as anti-collagenases weren’t effective. Some researchers showed improving results by using TGFβ1 suppressors such as losartan, in order to reduce fibrosis in affected skin [7]. Usage of colchicine in epidermolysis bullosa acquisita resulted in favorable clinical outcomes [23]. Skin blisters firstly should be lanced, evacuated and then dressed by a non-adherent covering material and also a padding for maintenance of stability and integrity. Growth retardation in children with EB should draw attention to consider the fluid and electrolyte balance in those patients and requirement of nutritional support. Blood transfusion and iron supplementation are main options to cure anemia in these patients. Other nutritional supplementations for these patients consist of calcium, vitamin D, carnitine, selenium and zinc. Prevention from hand contracture could be acquired by occupational therapy. Surgical therapies including repeating release of fingers from each other, gastrostomy (by surgical intervention), esophageal dilation is demanded, endoscopic dilation by means of balloon catheter or bougie and in more severe cases, usage of gastrostomy tube are recommended as the main treatment options [7].

5. Genetic mutations in different types of EB

1) Simplex epidermolysis bullosa

Genetic disturbances in keratins 5 & 14 genes are present in this type of disease [25]. Furthermore mutations in genes responsible for expression of intracellular domains of BPAG1 protein has been also reported [26]. Mutations in CD151, a stabilizer component of hemi desmosome structure which supports constructional placement of integrin alpha 6 beta 4, also have been suggested as the etiological genetic disturbance in this type of disease [27]. A new mutation also recently reported to be associated with simplex type of epidermolysis bullosa; This mutation occurs in the initiation codon of kelch-like 24 protein and results in a degradation-resistant N-terminally truncated kelch-like 24 protein. Kelch-like 24 protein is suggested to play role in the turnover of intermediate filaments especially keratin 14 in keratinocytes [28].

In EB simplex with muscular dystrophy, mutations in genes responsible for the expression of plectin, a hemidesmosome subunit, have been suggested in this type of disease [8, 29]. In a previous study, mutations applied on plectin genes resulted in manifestations of epidermolysis bullosa simplex with muscular dystrophy, proved the responsibility of this gene in pathophysiology of this type of disease [30].

2) Junctional epidermolysis bullosa

The etiological factors proposed for junctional EB contains of mutations in the genes encoding LAM B3 chain of laminin-332 [9]. The old names of laminin-332 were ‘laminin 5, nicein (BM-600), Kalinin and epiligrin [31,32]. It has been indicated in previous studies that, some of subunits of cellular hemi desmosome complex including, laminin 5 (epiligrin / Kalinin / nicein) and integrin beta 4 are disturbed in this type of EB leading to lack of formation of hemi desmosome structure [33-36].

In generalized benign atrophic type of junctional epidermolysis bullosa, mutations in another hemi desmosome subunit, bullous pemphigoid antigen 2 (BPAG2, BP 180 or collagen 17) is reported as an etiological factor [37].

In a previous research, mutation applied on the intracellular domain of integrin beta 4, resulted in disconnection between integrin beta 4 and intracellular keratin plexus, manifested the clinical features of junctional epidermolysis bullosa. This result proof the role of integrin beta 4 as an etiological factor of EB [38].

3) Dystrophic epidermolysis bullosa:

In this type of disease, mutation in exon number 64 of COL7A1 gene have been suggested among the Iranian population [10]. Mutation in collagen VII gene results in renal disorders, mesangial proliferative diseases, PSGN and renal amyloidosis accompanying by dystrophic epidermolysis bullosa [39]. (Figure 1)

4) Kindler–be syndrome

The main gene which its mutations take the responsibility of kindler–be syndrome is FERMT1 gene that encodes fermitin family homolog-1 (FFH1). This family play role as a focal adhesion molecule and an actin cytoskeleton component. 37 different loss of function mutations on FERMT1 gene have been identified in kindler–be syndrome [14].

Molecular and cellular intermediates in EB

Defects in hemi desmosome structure and formation:

Overall as mentioned previously, disturbances in hemi desmosome structure subunits are probable etiological factors of EB especially in junctional type. By electron microscopy studies, it has been proved that hemi desmosome structures appear in lower numbers at basal surface of keratinocytes of patients who suffer from junctional type of EB [33].

Subunits of hemi desmosome contains of plectin, integrin alpha 6 beta 4, BPAG1e or BP230 (epithelial isoform of bullous pemphigoid antigen 1), BPAG2 or BP180 (a transmembrane collagen protein) and CD 151 [40].

Integrin Alpha 6 beta 4 and BP180 are two hemi desmosome subunits establish the connection between ECM (extra cellular matrix) and intracellular keratin plexus (keratins 5 & 14, the main keratins in epithelial keratinocytes) [41].
The formation of hemi desmosome structures initiate by attachment of integrin alpha 6 beta 4 subunit to plectin [42] by mediation of fibronectin III subunit of integrin beta 4 [43], plays the role as the core of hemi desmosome structure formation. This connection make some changes in the molecular structure of both plectin and integrin beta 4, which consolidate the whole structure [44]. Subsequently, integrin beta 4 connects to other hemi desmosome subunit called BP 180 [42].

Integrin Alpha 6 beta 4 has four pair of connecting domains (fibronectin type III) for attachment to other subunits of hemi desmosome structure [45]. Related domains on BP180 connects to integrin alpha 6 beta 4 through the second pair of connecting domain fibronectin III on integrin beta 4, a connection which is necessary for the formation and strength of hemi desmosome complex [46-48].

The other hemi desmosome subunit BP 230, attaches on one hand to BP180 and from the other hand to intracellular cytokeratin filaments. BP 230 also connects to the forth pair of connecting domain fibronectin III of cytoplasmic terminal of integrin alpha 6 beta 4 in order to tighten the hemi desmosome structure more. BP 180 furthermore connects to integrin beta 4, BP 230 and plectin subunits [47].

The mutant type of integrin beta 4 especially in junctional type of EB, is inefficient in making connection with plectin and BP180 subunits, results in defects in hemi desmosome structure formation. However there is not any disturbance in connection of integrin beta 4 with its pair; alpha 6 integrin [45].

The spatial placement of integrin beta 4 as a transmembrane protein in the basolateral surfaces of keratinocytes cellular membrane, is regulated by a membrane surface protein called CD 151, which is known as the sponsor framework that supports spatial placement of the other subunits during hemi desmosome formation [49]. Furthermore, up regulation of integrin beta 4 results in higher expression of CD 151 [40].

Plectin as another subunit connects to intracellular keratin types 5 & 14 in keratinocytes through its connecting domains [50].

Integrin beta 4 also connects through its extracellular terminal to extracellular matrix protein, laminin 332 [40]. Therefore, the connection between intracellular keratin plexus and extracellular basement membrane establish by mediation of integrin beta 4 – plectin complex.

In previous studies it has been indicated that the absence of intracellular keratins leading to absence of integrin beta 4 through phosphorylation of beta 4 at ser 1354 and ser 1362 finally results in endocytosis and down regulation of integrin beta 4 and disjunction of beta 4 from plectin [51]. In other studies, EGF and PMA (phorbol 12-myristate 13-acetate) have been suggested as factors which phosphorylate integrin beta 4 at ser 1356 and ser 1364, result in a same output; disturbance in hemi desmosome formation and increase in cellular migration [52]. Hemi desmosome formation is not dependent to laminin-332 existence however plectin is a key component for hemi desmosome formation [53] and 1a isoform of plectin is more specific for hemi desmosome structures in keratinocytes [54]. Over all, the formation of this structure isn't dependent to extracellular domains and connections of integrin beta 4 (consist of connections with laminin 332 and alpha 1 subunit), however the intracellular connections of integrin beta 4 are vital in hemi desmosome structure formation. These intracellular domains are targets of mutations in epidermolysis bullosa disease [55].

In a similar way, BP 180 extracellular terminal is not vital in the formation of hemi desmosome while its intracellular domains are. Furthermore, integrin beta 4 regulates the spatial placement of BP180 at the basolateral surface of keratinocytes and the absence of beta 4, results in disarrangement of BP180 subunits placement over the cellular membrane surface [56].

**Other factors mediate EB**

Connections between epidermal keratinocytes establish by desmosome structures; when these keratinocytes become cornified, desmosomes modify and transform to corneodesmosomes. Defects in these connections result in epidermolysis bullosa and palmoplantar keratodermas disease [57].

Defects in desmosome structures are also suggested in epidermolysis bullosa disease. In lethal acantholytic epidermolysis bullosa, homozygous deletion at exon 20 of desmplakin gene, have been suggested as an etiology of the disease results in cardiomyopathy as a manifestation of LAEB [58].

Transglutaminases (protein glutamine-γ-glutamyltransfease, TGase, TG) consist of a family containing 9 different types of proteins with different roles in catalytic and also cellular signaling processes. The principal TG proteins types in epidermal layer consist of TG1, TG2 and TG3 and TG5, distributed diffuse in this layer of skin [59]. TG5 is present at the spurious and granular layers of epidermis and also in the basal layer and hair follicles.

In many previous studies, mutations in TG5 gene have been suggested as the cause of acral peeling skin syndromes and the absence of this protein resulting in a clinical situation resembling epidermolysis bullosa simplex [59-62].

Mutations in collagen VII gene and its absence in skin keratinocytes in dystrophic type of epidermolysis bullosa results in changes in micro environment of skin tissue leading to prolonged wound healing process with subsequent increased inflammation in those wounds and increased risk of development of squamous cell carcinoma in skin, as the main reason of death among these patients [63].

Oxidative stress and mitochondrial dysfunction play main roles in the pathophysiology of kindler-be syndrome. Oxidative stress indexes such as GSSG/GSH ratio (oxidized and reduced glutathione), MDA (malondialdehyde) and GCL (gamma-glutamyl cysteine ligase) show altered levels in this disease. Electron microscopy studies on keratinocytes in skin biopsies of these patients showed mitochondrial morphological abnormalities and derangements [64].

**Previous studies**

Hemi desmosome formation and Gene therapies of disturbed hemi desmosome subunits genes: Plectin isoform 1a is the most specific isoform presents in hemi-desmosome structures in human skin. In a research performed in 2003 at university of Vienna, after preparation of culture of keratinocytes...
involved by epidermolysis bullosa simplex with muscular dystrophy, accompanied by lack of plectin gene (PLECTIN -/−), researchers transfected those cells by plasmids PBK23 & PGR99 containing plectin 1a gene. Results showed the increase in number of hemi desmosome structures on cell surface to the normal values (even in some keratinocytes was higher than normal in number) and cellular connections established. Transfection of cells by other isoforms of plectin gene, didn’t resulted the same; indicates the specificity of 1a isoform in human keratinocytes. Furthermore, the expression of only intracellular terminal of plectin 1a was not enough to result in the same result and the expression of whole gene is recommended [54].

Furthermore in electron and fluorescent microscopy studies on keratinocytes with junctional EB; done by means of antibodies against intracellular cytokeratin filaments, results showed the normal values of intracellular keratins in comparison to normal keratinocytes. In junctional type of EB, hemi desmosome structures were down regulated to periphery of nucleus instead of basolateral membrane surface of keratinocytes [65].

In another study in 2014, researchers at Stanford University transfected stem cells of keratinocytes of a patient was suffering from dystrophic epidermolysis bullosa by means of lent viral vectors in order to conduct gene therapy for collagen 7, resulted in normal keratinocytes with potentials for repair and regenerate the skin [66].

Epidermolysis bullosa aquisita is another subtype of EB. Immunologic factors have been indicated as etiological factors in this type of the disease including Anti bodies against collagen 7. TNF alpha levels are also increased in the serum of these patients indicates the immunologic and inflammatory aspects in pathophysiology of this disease.

In an attempt to cure EB, in 2016 at University of Lübeck, German researchers used anti TNF monoclonal ABs and TNF suppressor drugs such as etanercept. Results showed decrease in amount of skin lesions and blisters [67].

In an effort in order to replace the gene of integrin beta 4, keratinocytes with junctional epidermolysis bullosa with pyloric atresia transfected by integrin beta 4 gene recombinant cDNA. This gene therapy resulted in orientation of plectin and BP180 toward basolateral surface of keratinocytes; However BP230 did not oriented like other subunits and remained diffuse in the cell membrane [45].

In a previous research keratinocytes of benign atrophic junctional epidermolysis bullosa which have had normal integrin beta 4 and plectin genes but mutant BP 230 AND BP180, transfected by recombinant cDNA of BP180 by means of viral vector (simian virus 40 (SV40)); resulted in expression of BP180 and its orientation and placing beside integrin beta 4 at basolateral surface of the cell membrane in hemi desmosome structures. Furthermore BP230 subunits oriented in basal surfaces of keratinocytes, aggregated at hemi desmosome structures [68].

**Stem cell transplantation and tissue engineering**

Stem cells give rise to any other types of cells in the body. Stem cells play major roles in repair of different organs; as an example they take part in gastrointestinal tract lining epithelium renewal continuously. Stem cells divide into two types. First, embryonic stem cells which originate from inner cell mass of blastocysts during embryonal development and second type, adult stem cells, located at different organs and play role in tissue repair.

Stem cells are also located at hair follicles and are multi potential and has the ability to give rise to many types of cells containing epidermal cells (keratinocytes). Hair follicle stem cells mostly reside at bulge region which is located between the opening of sebaceous gland and the attachment site of arrector pili muscle. These stem cells are important in compensating the cellular defects (due to lack of junctions and occurrence of wounds) in diseases such as epidermolysis bullosa. In fact among stem cells of skin tissue, hair follicle stem cells are more potential for replacing epidermal cells in diseases and injuries resulting in skin defect [69-71].

Hair follicle stem cells are suggested to be capable of repair of skin defects due to previous injuries and isolated hair follicle stem cells in a small biopsy are applicable to repair extensive full thickness skin defects. This autologous transplantation overcomes major challenges regarding the use of stem cells in clinical application, because it avoids immune reactions and graft rejection and ethical concerns [72].

In a previous study, patients with recessive dystrophic epidermolysis bullosa (RDEB) have been injected subcutaneously and intravenously by a combination of keratinocyte-derived pluripotent stem cells and stem cells from RDEB patients skin (KC-iPSCs-MSC) converted to mesenchymal stromal cells. Those stem cells have been cultured in a media containing 6-bromoindirubin-3’-oxime, activin A and bone morphogenetic protein 4. Results showed improvements in healing process of skin in RDEB knocked-out (immune-deficient) Mice and increase in type 7 collagen presentation at dermoepidermal junction surface of new proliferated keratinocytes. Those pluripotent stem cells can obtain from bone marrow and even lonely implanted in skin tissue results in improves in clinical outcomes of those patients [73,74].

Human embryonic stem cells (hESCs) derived keratinocytes are suitable alternatives for traditionally used immortalized cell lines (i.e. HaCaT cell line) for stem cell mediated treatment of skin wound. In a previous study regarding mechanical stress effects on hESCs it has been clarified that mechanical stress results in increase in expression of some genes including matrix-metallopeptidase 9, keratinocyte growth-factor-receptor, connexin43, catenin β1, endothelin1, integrin α6, desmoglein 1, interleukin α1, E-cadherin, keratin1, 6, and 10 and laminin α5 in both cell line types mentioned above. hESCs derived keratinocytes express matrix-metallopeptidase gene earlier in comparison to HaCaT stem cells in response to mechanical stress. So hESCs response characteristics are more resemble to that of normal human skin and so they are more suitable for stem cell assisted treatment of skin diseases [75].

In 2015 an experimental study on mice models of RDEB investigated the intrahepatic injection of cord blood derived stem cells; resulted in improvement of disease phenotypes [76].

Placenta (Placenta derived stem cells (PDCS)) and fetus cord (cord blood derived stem cells) are sources for obtaining stem cells in cellular treatment of RDEB. Because of homogeneity of
stem cells antigens and recipient antigens, immune reactions are not expected. Researchers used these stem cells for treatment of RDEB in col7a1 gene knocked-out mice. Results showed increased junction between dermis and epidermis at dermo-epidermal junction (DEJ) [77].

Stem cell cultures containing normal keratinocytes are applicable to wounds of patients with junctional epidermolysis bullosa to compensate the skin defects [78].

Transplantation of bone marrow from human to mice through injection into venous blood (in a mice with epidermolysis bullosa and defect in collagen 17) resulted in compensation of collagen 17 defect in hemi-desmosome structures and improvement in clinical outcomes. It has been clarified also that CD34+ cells have the potential to replace keratinocytes in the skin of patients suffering from epidermolysis bullosa [79].

Researchers also applied gene therapy over iPSCs to replace defect of collagen 7 gene in patients with RDEB. First, pluripotential stem cells obtained from patients with RDEB, then those cells transfected by type 7 collagen gene (col 7a1) in vitro and then genetically manipulated (corrected) stem cells expressed col 7 [80]. Hematopoietic cell transplantation (HCT) results in replacement of defected cells with normal ones, improving the patients’ clinical outcomes [81].

Application of decidual stromal cells (DSC) as allogenic mesenchymal stromal cells (MSCs) are also effective in patients with generalized severe junctional epidermolysis bullosa (or previously called Herlitz JEB). Transient healing of wounds occur after treatment by these stem cells replacing defected skin. Alloimmunization is a side effect of stem cell therapy which can result in clinical conditions like autoimmune cystitis or even graft versus host disease [82].

Transplantation of stem cells obtained from bone marrow or peripheral blood also can improve the clinical outcome in patients with junctional epidermolysis bullosa [83].

Exogenous induction of mesenchymal stem cells by TGF-β and TNF-α for 48 hours, resulting in 8 – fold increase in col 7a1 expression and so increase in collagen 7 production in those cells and also increases the tsg-6 expression (resulting in wound healing acceleration and immunosuppression). Induction of these cells by SDF-1alpha increases the expression of CXCR4, tsg-6 and col 7a1 genes [84]. So when it comes to application of stem cells in vivo in order to improve wound healing process in destructive skin diseases such as EB, the molecular intermediates and induction factors should be considered in order to accelerate the process of cellular differentiation and wound healing.

Subcutaneous daily injection of G-CSF stimulates and recruits stem cells of skin. This treatment in patients with dystrophic epidermolysis bullosa results in decrease of lesion size and skin blistering [85].

Epithelial stem cells (ePSCs) can also arrange in cultured epithelial cell sheets (CES). CES has the potential to compensate skin defects by transplantation of cultured cell sheets over the lesion area in patients with epidermolysis bullosa [86].

Combination of tissue engineering techniques and gene therapy by viral vectors have been also suggested as a treatment option for dystrophic type of epidermolysis bullosa. During this procedure, patients’ stem cells obtain by biopsy from skin or from peripheral blood. Transfection of stem cells by viral vectors to replace defected col 7a1 gene is the next step in the treatment. Finally transfected and genetically modified stem cells will transplant back to the skin in order to compensate absence of collagen 7 in skin, leading to improve in clinical features [87].

The latest treatment mentioned as the combination of tissue engineering and gene therapy is also effective in patients with simplex epidermolysis bullosa to restore skin integrity by correction of genetic mutations in keratinocytes [88].

In a previous study in 2018 at university of Milan, usage of allogeneic cord blood derived platelet gel obtained from a term, healthy neonate used to repair the mechanobullous skin ulcerations in patients with epidermolysis bullosa, resulted in promising clinical outcomes [89].

An Italian research group in 2006 successfully generated ex vivo, gene corrected, epidermal sheets obtained from a patient with junctional epidermolysis bullosa and utilized it as a skin graft resulted in improvement of clinical features [23].

**Discussions**

As mentioned above, in epidermolysis bullosa, the targets of mutations are consisted of some of hemi desmosome structure subunits including integrin beta 4, plectin, BP 180 and keratins 5 & 14. Resulting from electron microscopy studies, the absence of hemi desmosome structures at basolateral membrane of keratinocytes is more prominent in junctional type of epidermolysis bullosa and is the main etiological factor in these patients [90].

From the previous studies we conclude that, hemi desmosome subunits have reciprocal supportive effects on each other’s spatial placement and up regulation at keratinocytes membrane surfaces to maintain and support the hemi desmosome structure. These interactions including the moderator effect of BP 180 and intracellular keratins 5&14 on integrin beta 4 and also the reciprocal supportive effect between integrin beta 4 and CD151. These supportive effects of subunits on each other, indicate the mathematical and logical relationships in the form of certain ratios between the number of each subunit at keratinocytes membrane surfaces and their concentrations in tissues resulting in arrangement of subunits and formation of hemi desmosome structures.

In many previous efforts in order to perform gene therapy for these mutant genes of hemi desmosome subunits in epidermolysis bullosa patients, researchers have not considered the previous mentioned point, and gene therapies have cared about just one of the subunits, however others are disturbed too and a single gene therapy, while the other genes are mutant and them absence down regulates our target gene, would not be sufficient for treatment.

Another criticism against those studies which spend to gene therapy for treatment of EB is about the type of vectors we use. Most of the researchers use viral vectors for gene therapy while they’re not appropriate for application in human species. One of the non-viral vectors suggested for gene therapy is HPAl (highly branched poly (β-amino ester)) which is viable and suitable for topical or subdermal usage on the skin of patients affected by epidermolysis bullosa.
Tissue engineering techniques such as stem cell transplantation are also among treatment options. Stem cells can obtain from bone marrow, peripheral blood, umbilical cord, Placenta and etc. transplanted stem cells from a healthy individual regenerate skin tissue and accelerate healing of skin lesions. The combination of stem cell transplantation and gene therapy consists of in vivo correction of the mutation and defected gene of EB patient stem cells. Then transplanted stem cell transplants back to the skin to regenerate skin tissue.

Conclusion

Tissue engineering and gene therapy together are novel suggested treatment options for EB patients, but there is some obstacles to obtain a certain treatment by gene therapy over stem cells in EB. Application of viral vectors in human species are among restrictions of gene therapy due to ethical issues in EB patients because of risk of spread of and infection by viruses and unknown complications. Usage of non-viral vectors leading to higher applicability of gene therapy in EB patients. Author suggests from previous researches that there is a proportional and mathematical relation between number of each different subunits of hemi desmosome in its structure, which has been neglected in previous gene therapy researches and those proportions should be considered in gene therapy researches. In vitro gene therapy of stem cell keratinocytes and transplant them back to the skin is expected to improve the clinical features in EB patients.

Conflicts of Interest

The authors indicate no potential conflicts of interest.

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