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Dynamic Action of Myofibroblasts in Skin and Bone Marrow Transplantation

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Abstract

Skin is highly accessible and evaluable organ, which accelerate the understanding of novel medical innovation in association with organ transplantation, engineering, and wound healing, as well as the stage-specific adaptability of transplanted Bone Marrow (BM) cells. In skin transplantation biology, multi-stage/-angle damages occur in both grafted donor and perilesional host skin, and need to be repaired properly for the engraftment and later maintenance of the local homeostasis and characteristic skin architecture, such as stratified squamoid epithelium and dermal components. These local events are more unlikely to be regulated by the host immunity, because the donor (allogenic) skin engraftment mostly accomplishes onto the immunocompromised or immunosuppressive animals. Accumulating evidences have emerged the importance of alpha-Smooth Muscle Actin (SMA)-positive myofibroblasts, via stage- and cell type specific contribution of TGF-beta, PDGF, ET-1, CCN-2 signaling pathways and/or mastocyte-derived mediators (e.g. histamine and tryptase), for the functional reorganization of the grafted skin. Moreover, particular cell lineages from BM cells have been shown to harbor the differentiation capacity into multiple skin cell phenotypes, including epidermal keratinocytes, and dermal endothelial cells and pericytes, under controlled by chemokines or cytokines, but the trans-differentiation into alpha-SMA+ myofibroblasts is possibly reversed by inactivation of MEK/ERK signal cascade. We review the recent update of the myofibroblast biology in association with the reconstitution of the engrafted skin, and also work on translating this attractive action into the application of BM transplantation medicine in genetic skin diseases.

Keywords: Myofibroblasts; Alpha-smooth muscle actin; Bone marrow transplantation; Skin graft; Genetic skin diseases; Mesenchymal stem cells

Introduction

Skin represents a substantial part of mammalian ectoderm, which is normally exposed by various exogenous stimuli, for example, UV irradiation, infection, temperature, moisture, and mechanical stimuli [1]. Because skin is highly accessible to any of diagnostic and treatment procedures, researches for skin transplantation, as well as skin engineering and wound healing, can accelerate the understanding of underlying pathophysiology for the novel medical innovation utilizing self-made or more feasible skin equivalents. Over a thousand gene mutant loci for inherited human disorders have been reported thus far [2], and approximately one third of these disorders exhibit the corresponding skin abnormalities, in which the gene targeting/molecular-based therapies have yet to be standardized practically. Skin transplantation strategy and its relevant technology may thus retain the potential benefit in such skin conditions.

Histologically, the predominant cell populations in mammalian skin comprise dermal fibroblasts and epidermal keratinocytes. Both cells show different morphology and function, and are separated structurally by basement membrane. Ours and other studies have utilized two-/three-dimensional co-culture or complex "organotopic" culture systems, allowing to address the importance of paracrine interaction between fibroblasts and keratinocytes [3,4]. Upon these in vitro skin equivalent assays, little is known about how these two cell types are reorganized properly in the grafted skin. The site of the skin graft might eventually account for multi-stage (acute/chronic) and multi-focal damages of the donor and perilesional host skin, micro-hemorrhage/exudation, and later excess fibrosis in the dermis. The grafted skin therefore needs to be repaired and reconstituted through these inevitable events. More specifically, dermatologists have much interests to know how the biological architecture characteristic for the skin (e.g. stratified squamoid epithelia, dermis intermixed with extracellular matrices and dermal components, and their polarity) can be maintained after the skin transplantation. One may consider that the particular cell phenotypes play a central role in the orchestration of the skin reconstitution, and if so, under what particular circumstances for this process? The chain of these biological events is more unlikely to be regulated by cellular and humoral immunity in the host, because in vivo researches for human skin transplantation has accomplished the donor skin allograft onto the immune compromised animals, such as nude and athymic mice, or those treated with immunosuppressive agents or the particular T cell subset (CD4+CD25+Foxp3) [5,6]. Inversely, a somewhat study limitation may thus often enable us to access to the insight associated with the skin transplantation immunobiology.

For understanding the cell-specific action in the skin transplantation, evidences from BM transplantation study may in part bring the clue. Native BM cells comprise the substantial proportion of cell sources that play a pivotal role in tissue homeostasis, repair, and regeneration. These cell populations are originated from either hematopoietic or mesenchymal stem cells, and subpopulations that are capable of differentiating into multiple cell lineages [7,8]. A series of recent research progress have emerged that BM cells can provide not only fibroblastic cells, but also epithelial cells in the lung and intestinal epithelium, and skin [9]. Particularly in skin, a transplantation of sex (XY chromosome)-mismatched BM cells or intrinsically labeled BM

cells has demonstrated that keratinocyte specific marker-positive BM cells appeared in the epidermis, hair follicles, and sebaceous glands [10-15]. Moreover, in patients who underwent BM transplantation, donor BM cells displaying wide-ranged keratinocyte markers (pan-keratin) were detectable in the epidermis and maintained for over 3 years after the transplantation [16]. These data series suggest that the trans-differentiated keratinocytes from BM cells not only aid the impairment of the residual epidermal function after transplantation, but also participate in the compensation of the epidermal circumstances at the affected skin sites. On this basis, the population of BM-derived keratinocytes is secured functionally and structurally as a baseline stable supply. However, it remains unclear: i) how the BM cells are recruited strictly into the grafted skin, and if once they failed this process, how it can be corrected properly, ii) how the recruited BM cells contribute functionally to the local skin regeneration, and more interestingly, iii) whether the newly established epithelial-mesenchymal interaction can maintain the local skin homeostasis analogous to the host skin. From a dermatological view point, this review focuses on these attractive points in association with the cell type-specific reorganization in the skin transplantation, particularly dynamic action of myofibloblasts, as well as the relevant molecular profiles. These advanced evidences may help to ask how we can establish and refine the better medical approaches for the persistent skin wound condition, particularly in genetic skin diseases.

Biological Action of Myofibroblasts in Skin and BM Transplantation

After skin transplantation, the grafted skin sites need to repair some inevitable minor trauma and inflammation, for example, occasional hemorrhage caused by microvascular damage, exudative stress (edema), later excess micro-fibrosis, or even focal necrotic changes, in order to adapt to the host skin circumstance. At the early stage of these minor tissue damages, the grafted donor skin and/or perilesional host skin can primarily drive the recruitment of the particular subset of fibroblastic cells, termed "myofibroblasts" that specifically express the intracellular structural protein α-SMA [17]. α-SMA is strictly expressed in cells of the smooth muscle lineage, promotes stronger force generation compared with other actin isoforms in fibroblastic cells, and thus plays a pivotal role in the cell migration and local tissue contractility [18]. Despite its convenience detectability and unique marker for myofibroblast phenotype [19], the most important defining feature of myofibroblasts is the de novo development of stress fibers and contractile force and, questions remain to be ascertained the potential significance of other candidates for the myofibroblastic markers, such as endosialin [20], P311 [21], integrin α11β1 [22], osteopontin [23], and periostin [24].

Myofibroblasts can migrate into the grafted skin, and subsequently produce collagens, fibronectin, and proteoglycans to reconstitute the local extracellular matrix (ECM) network in the dermis [25,26]. During this process, αSMA is reorganized into the complexes of stress fibers for biological connecting to the surrounding ECM molecules, and participates in the exert contraction and mechanical tension, as well as reconstitution of primary intra/intercellular skeleton, for the establishment of the functional remodeling/framing of connective tissue. In contrast, persistence and/or aberrant increase of the local myofibroblasts and its action may be responsible for fibrosclerotic skin diseases, such as systemic scleroderma, morphea (localized scleroderma), or hypertrophic scar [27]. Another in vitro observation with human embryonic stem (hES) cells utilizing a three-dimensional skin model has shown that hES cell-derived mesenchymal cells that constitutively express α-SMA can promote multi-layered epithelium and the resultant wound healing process, with increased production of hepatocyte growth factor HGF, an essential factor for skin development and repair [28,29]. This characteristic cell phenotype may also be analogous to myofibroblastic cell lineage, with possible implication of epidermal-mesenchymal cross talk in a HGF-dependent manner.

The local myofibroblasts – without regard to the cells that recruited eventually into the donor grafted skin or local residential cells – are considered to be originated from multiple cell sources in vivo. Current concept favors the presence of at least three distinct cell sources for skin myofibroblasts (Figure 1); i) BM-derived mesenchymal stem cells, ii) pericytes that composed of skin microvasculature, iii) resident fibroblasts in the donor grafted skin and/or the perilesional host skin when grafted the skin [17]. These three myofibroblast sources are selective and transformed appropriately in skin damage- andhealing stage-dependent manners [30,31]. On this basis, fibrosclerotic diseases may at least in part share the pathogenic imbalance in myofibroblast recruitment and clearance. However, there have been no convincing data for what percentage of the particular cell lineage-derived myofibroblasts is involved in the reconstitution of the skin engraftment. Also, little is known about whether any biological thresholds of the myofibroblast recruitment exist in this event. For this detection, combination of any other characteristic marker(s) may be needed.

Molecular-dependent Differentiation and Reversal of Myofibroblasts

There have been extensive reviews on the signaling cascades for transforming growth factor (TGF)-β, platelet-derived growth factor (PDGF), endothelin (ET)-1, cysteine-rich protein 61/connective tissue growth factor/nephroblastoma overexpressed gene (CCN)-2, and several soluble mediators from mastocytes and the potential contribution of this pathway in the myofibtoblast biology [27,32,33] (Figures 1 and 2). Each of these supports a variety of biological action associated with trans-differentiation and reversal of myofibroblasts, and is most likely to make a complex interrelationship between myofibroblasts and local ECM molecules to promote the skin wound repair, remodeling, and reorganization after transplantation.

Five TGF-β isoforms, TGF-β1-5, exist in mammals and are generated initially as biologically latent precursors, enabling them to bind to a heteromeric receptor complex (a type I and II receptor complex) [34]. The former receptor phosphorylates Smad2 and 3, which subsequently binds to Smad4, and finally activates the transcription of the corresponding genes in fibroblasts. Activation of the TGF-β signaling increases the production of collagen I and ECM molecules [35,36], in parallel with CCN overexpression [37], finally causes α-SMA expression and α-SMA-dependent stress fiber formation in resident fibrocytes and naive fibroblasts [38-41].

There are 3 isoforms of endothelin, ET-1, -2 and -3 [42]. ET-1 is the major isoform in human and is produced by various cell types, including endothelial cells, BM cells, hematopoietic cells, cardiomyocytes, and fibroblasts. ET-1 is secreted as a 212-aminoacid precursor (prepro-ET-1) and enzymatically cleaved to a biologically active 21-aminoacid peptide, which can bind to the two distinct receptors (ET-A and ET-B). ET-1 induces – in cooperation with TGF-β pathway – myofibroblast formation and migration, and ECM

contraction via binding to ET-A/-B receptors and the resultant activation of downstream signalling molecules, Akt/rac [42,43]. A clinical observation from the treatment with bosentan, an ET receptor antagonist, for patients with systemic sclerosis suggests the direct effects of ET in the peripheral circulatory systems and its surrounding connective tissue [44,45].

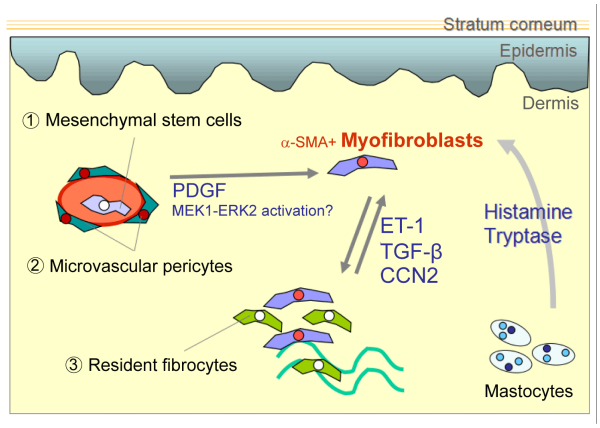

Figure 1: Scheme of myofibroblast differentiation in skin. The local myofibroblasts characteristic for α-SMA expression are originated from multiple cell sources in the skin, and nominated from at least 3 distinct cell sources; BM-derived mesenchymal stem cells, microvascular pericytes, resident fibroblasts in the donor skin graft and/or in the perilesional host skin (after the skin graft). Some molecules can organize the cell-type and tissue-specific differentiation into dermal myofibroblasts.

CCN2, a member of the CCN family of matricellular proteins, is induced by TGF-β and ET-1 system, vice versa, and is therefore considered an essential cofactor required for the particular subsets of TGF-β cascade, FAK/Akt/PIP3K [42,46]. CCN2 can activate the fibrotic phenotype of cells, and also support a variety of biological TGF-β action, such as type I collagen synthesis, α-SMA expression, and promotion of cell-ECM interaction [47,48].

PDGF family members include PDGF-AA, -AB, -BB, -CC and –DD, and bind with two different PDGF receptors α and β [49]. PDGF can enhance multiple cell types, including neutrophils, macrophages, fibroblasts and smooth muscle cells, to proliferate and migrate them into the skin wound, and also stimulate the differentiation into myofibroblasts, thus contributing to the local skin remodeling and contracture [50]. Mice treated with imatinib mesylate, a PDGF receptor-specific tyrosine kinase inhibitor, exhibited delayed skin wound healing with decreased levels of the local myofibroblast number, collagen type I expression [51], and non-canonical TGF-β signal network [52,53]. The dynamics of the multiple molecular interactions suggest the prominent biological action of PDGF in the stage-specific regeneration of skin. Also, a recent study has suggested the potential contribution of a subset of PDGF receptor α-positive BM cell population in the epidermal keratinocyte differentiation and reorganization in mice skin [30].

Mastocytes have pleiotropic action for fibroblast biology by secreting a variety of chemical mediators and cytokines. In cell co-culture and skin equivalent culture systems, for example, human mastocyte line HMC-1 cells can induce the expression of α-SMA [54]. This induction is mediated by a paracrine action of histamine and a serine protease tryptase, thereby contributing to the fibroblast-dependent skin contraction.

In contrast to the cell source-specific molecules for the trans-differentiation toward the myofibroblast phenotype, this cellular event may be reversible by downregulation of MAPK/ERK kinase (MEK)1 and/or ERK1 and 2 pathways [55].

Mesenchymal Stem Cells in Skin Transplantation

Differentiation into keratinocytes and its progenitor or stem cells

Accumulating evidence has gained the possibility that mesenchymal stem cells (MSCs) can contribute to the skin wound repair and development. For example, infusion of genetically engineered green fluorescent protein (GFP)-expressing BM cells into mice utero results in accumulation of a certain subpopulation of GFP-positive cells in non-wounded skin dermis, particularly in highly association with hair follicles [56]. More precisely, in vivo transplantation of sex (XY chromosome)-mismatched human BM cells or GFP-expressing murine BM cells has demonstrated that at least by 4 weeks after the transplantation, keratinocyte marker-positive BM cells appeared in the epidermis, hair follicles, and sebaceous glands [10-15], sites that harbour skin stem cell niches [57] (Figure 2). Thereafter, the locally recruited BM cells into the grafted (damaged) skin in mice can be maintained at least 5 months [30]. Considering the short turnover time of mice skin (2-3 weeks), the long-residing BM-derived epithelial cells are most likely to contain subpopulation(s) of epithelial progenitor/stem cells. This characteristic cell population constitutively expresses PDGF receptor α, but neither c-kit nor Sca-1, and the differentiation activity is accelerated by a paracrine action of heparin-binding molecules from the skin graft, especially high mobility group box 1 (HMGB1) [58] (Figure 2). HMGB1 is an evolutionarily well-conserved protein, and is produced constitutively in various types of cells, particularly damaged and injured cells. It acts a DNA binding core in assembly of nucleoprotein complexes for the maintenance of nucleosomal structure and regulation of the corresponding gene transcription [59]. Infection and injury converge on common inflammatory responses that are mediated by HMGB1 secreted from immunologically activated immune cells, including macrophages/monocytes or passively released from pathologically damaged cells. However, mice and human BM transplantation studies have revealed that BM-derived keratinocytes account for an extremely rare population in both wounded and non-wounded skin epidermis; e.g. almost undetectable levels or only less than 0.0003% of all keratinocytes in the mice epidermis [31] and 0.14% of those in human epidermis [15]. These poor cell numbers are in agreement with the preliminary observation of recent reports, and in parallel, they never aggregate in the epidermis but mostly present therein as a single cell [15,30]. Conceptionally, the relatively scarcity of such cells may therefore raise questions about their biological significance in the skin engraftment. Besides, the recruited BM cells can be a potential source for supplying skin structural molecules, such as type VII (COL7) and type XVII collagens (BP180), both of which are essential anchoring molecules in dermal-epidermal junction (Figure 2). Loss-of-function mutations of these genes causes subtypes of genetic skin fragility and scarring diseases, recessive dystrophic (RDEB, OMIM #226600) and junctional epidermolysis bullosa (non-Herlitz JEB, OMIM #226650), respectively [60,61].

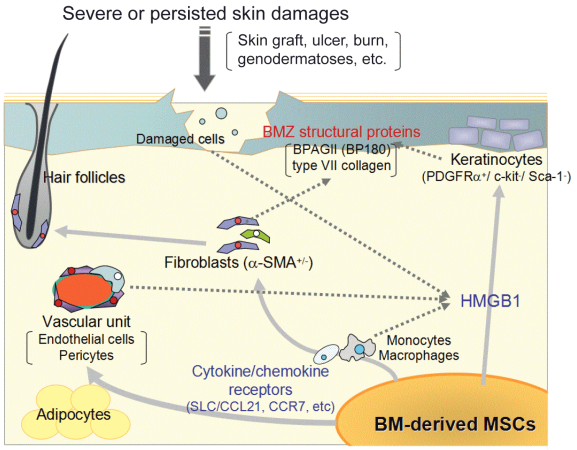

Figure 2: Trans-differentiation of BM-derived MSCs into the multiple skin component cells. The particular subset(s) of allogenically transferred MSCs, a PDGFR+/c-kit-/Sca-1-lineage, can differentiate into the keratin marker positive-epidermal keratinocytes via a paracrine action of HMGB1. In another cascade, the trans-diferentiation activity of the MSCs into other skin components, such as vasculature (endothelial cells and pericytes), follicular epithelium, and dermal interstitial fibroblasts, albeit much lesser with monocytes, macrophages, and adipocytes, is accelerated by certain cytokines/chemokines, especially CCR7-SLC/CCL21 pathway. These BM-derived multiple cell lineages can be a potential source for supplying skin structural molecules, such as type VII collagen (COLVII) and type XVII collagen (BPAGII; BP180), both of which are essential anchoring molecules in the basement membrane zone (BMZ).

Embryonic and postnatal transplantation of BM cells into mice lacking type VII or XVII collagens can successfully ameliorate the persisted skin wound and fragility by newly generation of the defected skin molecules [56]. Most convincing evidence from a clinical trial of allogeneic whole BM transplantation in a patient with RDEB has successfully shown that BM cells can repair the skin wound and restore the defected COL7 expression in the skin basement membrane zone [16]. Overall, these data suggest that minimally trans-differentiated BM cells are indeed sufficient for the generation of deficient skin protein(s) and restore the fragile skin condition in vivo.

Differentiation of BM cells into multiple skin cells

Along with a streamline for the functional epidermal differentiation of BM cells, a most recent investigation has explored that BM-derived MSCs intravenously injected can differentiate into multiple skin cell lineages, including epidermal keratinocytes, and dermal endothelial cells and pericytes, finally contributing to skin wound repair in mice, suggesting upregulation of angiogenic properties in the host skin [15] (Figure 2). This MSC phenotype harbors several chemokine receptors, especially CCR7, a receptor of SLC/CCL21 that enable MSCs to migrate into the local tissues [62,63]. Perilesional skin injection of SLC/CCL21, but not thymus and activation-regulated chemokine (TARC), can increase the baseline differentiation of MSCs into the wounded skin, resulting in the wound closure. In this study, the trans-differentiation activity of bulk MSCs into multiple skin cell phenotypes seems higher comparative with previous reports; ~0.14% of GFP-positive MSCs into epidermal keratinocytes, ~13.2% into endothelial cells and ~33.0% into pericytes in the dermis, albeit much lesser with monocyte/macrophage and adipocyte lineages [30]. Interestingly, the recruitment of BM-derived cells is significant in the grafted skin and long-standing damaged skin, being a similar condition to RDEB [56,64], whereas it is much lesser or almost negligible levels in most of transiently established skin wound healing models [30,31]. The proportion of the recruited and/or trans-differentiated BM cells seems considerably variable by the skin damage and its period. Another angle of evidence suggest that transplanted BM cells can attenuate the proliferation and differentiation of α-SMA+ myofibroblasts, as well as the aberrant constitution of the local extracellular matrices, via down-regulation of TGF-β and type I collagen [65]. This local reaction further enhances the expression of matrix-degrading zinc-dependent enzymes, matrix metalloproteinase (MMP) family members (MMP-2, -9 and -13), presumably contributing to the inhibition of skin fibrosis/scar formation [66].

Current Issues and Perspective

Despite the recent dramatic progress in the skin transplantation and wound healing studies, we face to some inconclusive debates that need to be addressed; how much of the trans-differentiation activity of BM-derived MSCs is indeed influenced by different characters in individuals, e.g. age, medical history and on-going treatments, and affected skin sites. Are there any biological thresholds to recruit MSCs or to induce α-SMA+ myofibroblasts for the proper skin engraftment and wound healing, if any, how can we analyze and standardize them? Which soluble molecules or combination of these (e.g. SLC/CCL21, HMGB1, and PDGF; Figure 2) – if add exogenously - are more efficient to ensure the favorable outcome of the stage-specific events in the transplantation? Particularly in the allogenic BM transfer, do these supplemental additives affect the baseline incidence of life-threatening complications, such as GvHD? These parameters should be estimated precisely and translated into the lack-of-functional protein genodermatoses and post-BM transplant complications.

Summary

Skin and BM transplantation researches have come to be saturated gradually by multi-angle evidence and interpretation from the relevant organ transplantation, and provide novel therapeutic implications. BM-derived cells with pluripotent differentiation capacity into multiple skin components, including myofibroblasts that have a potential capacity for trans-differentiation and reversal, may thus act as target cell(s) for the innovative molecular therapy for persisted skin wounds in genetic and autoimmune diseases.

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