Hypertrophic Scarring: Current Knowledge of Predisposing Factors, Cellular and Molecular Mechanisms

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Hypertrophic scarring (HSc) is an age-old problem that still affects millions of people physically, psychologically, and economically. Despite advances in surgical techniques and wound care, prevention and treatment of HSc remains a challenge. Elucidation of factors involved in the development of this common fibroproliferative disorder is crucial for further progress in preventive and/or therapeutic measures. Our knowledge about pathophysiology of HSc at the cellular and molecular level has grown considerably in recent decades. In this article, current knowledge of predisposing factors and the cellular and molecular mechanisms of HSc has been reviewed.

Skin is the largest organ of the body with various vital functions and a significant role in individual's self-esteem. The exposed nature of the skin to the external environment makes it vulnerable to injuries with variable intensities and depth. In postnatal life, while minor abrasions of the skin completely regenerate without scarring, deeper insults usually provoke a repair process which involves the proliferation of fibroblasts, deposition of fibrotic tissue, and ultimately scar formation. Disruption of the normal healing process may lead to chronic nonhealing wound at one end of the wound healing spectrum, or excessive fibrosis and hypertrophic scarring (HSc) at the other end. HSc often occurs after injuries to the deep dermis.¹ This pathologic scarring mainly occurs after burn injuries compared to other traumatic lesions or surgery.² For instance, nearly 70% of patients who survive severe burn injuries will develop HSc and suffer from sustained physical and psychosocial consequences.³

Creation and implementation of effective preventive or therapeutic interventions depends on clear and in-depth knowledge of factors involved in the development of HSc. Data collected from clinical studies have increased our ability to predict the possibility of abnormal scarring to a great extent. In addition, advances in basic research have elucidated some of the underlying cellular and molecular mechanisms contributing to HSc and have set the stage for the development of more effective preventive or treatment option(s) for this devastating fibroproliferative disorder.

Here, the current information about the predisposing factors such as individual characteristics, the extent and depth of injury, the effect of bacterial colonization, and time to heal, in addition to cellular and molecular mechanisms involved in pathophysiology of HSc are reviewed.

PREDISPOSING FACTORS

Although the factors influencing hypertrophic scar formation are not completely understood, several features have been identified as predictors of severe scarring following burn injuries. These features can be classified into two categories: intrinsic (patient related), and extrinsic (injury related) factors (Table 1). Among the intrinsic factors, female gender, younger age (≤ 30 years), darker skin type (Fitzpatrick skin type IV–VI), and anatomical site of burn (neck, chest, and upper limb) have been reported to be associated with HSc.^{2,4} Based on previous studies, the race of patients is another influential factor. HSc is more prevalent among Black/African American and Asian patients.^{5,6} Although the increased risk of postburn HSc in dark-skinned racial/ethnic groups suggests a genetic predisposition, the genetic determinants of HSc have not been identified yet. Genes involved in skin pigmentation such as melanocortin 1 receptor (MC1R) gene and its association with HSc have been studied recently. MC1R is a G-protein-coupled receptor that binds to α -melanocyte-stimulating hormone (α -MSH), one of the derivatives of proopiomelanocortin (POMC), and triggers dark eumelanin production by melanocytes.⁷ POMC is not only secreted by hypothalamus and pituitary gland, but is also produced locally by keratinocytes and melanocytes.⁸ Although the processing of POMC is incomplete in tissues such as the pituitary gland, human skin and hair follicle have the full potential to process POMC to its final derivatives.⁸ In addition to melanocytes, MC1R is also expressed on other cell types such as leukocytes, fibroblasts, and keratinocytes. It has been shown that α -MSH down-regulates the expression of proinflammatory cytokines in leukocytes, while up-regulating the production of interleukin-10 (IL-10), an anti-inflammatory cytokine, in human monocytes and keratinocytes.^{9–11} On the other hand, melanocortin signaling

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Table 1. Predisposing factors for HSc in burn patients

Intrinsic (patient related)	Extrinsic (injury related)
Female	Total body surface area burned (%TBSA)
Younger age (≤ 30)	Burn depth
Darker skin type (Fitzpatrick skin type IV–VI)	Surgical procedures (split-thickness skin graft)
Anatomical site of burn (neck, chest, and upper limb)	Time to heal >14 days
Race (Black/African American, Asian)	Hospitalization >14 days
	Wound complications (bacterial infection, graft loss, over granulation)

HSc, hypertrophic scarring; TBSA, total body surface area.
The information for this table was adapted from refs.^{2,4–6}

reduces the synthesis of types I and III collagen in dermal fibroblasts in response to transforming growth factor- $\beta 1$ (TGF- $\beta 1$) stimulation¹² (Figure 1). The spatial and temporal expression of the MC1R and α -MSH during cutaneous wound repair suggests that melanocortin signaling may have a role in wound healing.¹³ More than 80 variant alleles have been discovered for MC1R gene, including loss-of-function R163Q single-nucleotide polymorphism (SNP), which has been reported to have an allele frequency of 70% among East/Southeast Asians and 100% in Native Americans, who seem to be predisposed to HSc.⁷ It has been suggested that the loss-of-function SNP of MC1R might lead to increased risk of HSc among Asians and Native Americans. However, R163Q SNP was not present in Black/African Americans studied, who are at the greatest risk for hypertrophic scar formation.⁷

Extrinsic factors such as total body surface area burned (%TBSA), burn depth, multiple operations, meshed skin graft, time to heal, and bacterial colonization have been identified as predictors of severe scarring after burn in several studies.⁴ In a prospective case-control study among 616 adult acute burn patients in Western Australia, the authors found a strong correlation between %TBSA and the risk of developing raised scar. Compared to patients with 0 to 5% TBSA, the odds ratio of developing hypertrophic scar for patients with >5 to 10% and >10 to 15% TBSA was 2.77 and 4.92, respectively.⁴ Greater burn depth requiring surgical intervention such as split-thickness skin grafts versus conservative treatment was found to be another reliable predictor factor for HSc as indicated by previous studies.^{4,7,14,15} Wound complications such as wound infection, overgranulation, or graft loss also increased the risk of HSc significantly.⁴ According to previous reports, there is a strong correlation between the healing time and development of HSc.^{16–19} In a prospective study by Dietch et al. on 100 pediatric and adult burn patients with mean TBSA, 14% and 21%, respectively, followed from 9 to 18 months, the authors found that in cases that the burn wound healed between 14 and 21 days HSc developed in one third of the anatomic sites; while 78% of the burn sites became hypertrophic when the healing time was more than 21 days.²⁰ In another prospective study on 383 pediatric patients, the overall rates of HSc were 8.1% in those patients whose burn healed in less than 21 days and 56% in those who took longer than 21 days to heal.¹⁹ Taken together, shortening of the healing time is one of the factors that reduces the chance of hypertrophic scar formation.¹⁹

CLINICAL PRESENTATION AND HISTOLOGY OF HSC

Hypertrophic scars typically present as firm, red or pink colored elevations from the skin, which are commonly pruritic and remain limited to the borders of the initial wound.^{21,22,23} These scars emerge 4 to 8 weeks postinjury, followed by a rapid growth phase lasting up to 6 months, and gradual regression over a period of a few years.²² HSc is more common in anatomical locations with high tension, such as shoulders, neck, and presternum.²² Joint contracture is a common consequence of HSc.^{24,25} According to data from the National Institute on Disability and Rehabilitation Research Burn Model System database, for adult and pediatric burn survivors from 1994 to 2003, the most frequently involved joints were shoulder, elbow, wrist, knee, and ankle.^{25,26}

Histologically, the most common findings in HSc are flattened epidermis, replacement of the papillary and reticular dermis by scar tissue, which are primarily composed of fine, well-organized, type III collagen oriented parallel to the epidermis.^{22,27} Other characteristics of HSc include nodular structures containing myofibroblasts, collagen fibers arranged in whorl-like structures, and a prominent presence of vertically oriented blood vessels.^{22,27}

CELLULAR AND MOLECULAR MECHANISMS OF HSC

Role of Fibroblasts

Human dermal fibroblasts are the main type of cells responsible for the production and organization of the extracellular matrix (ECM) of the dermis. Since 1926 when the fundamental physiological properties of fibroblasts were described for the first time, a considerable body of research has been done on these connective tissue cells.²⁸ However, after almost a century, fibroblasts still remain a mysterious population of cells, mostly due to the lack of unique cellular markers and their diversity.²⁹

It has been previously shown that fibroblasts from different layers of normal skin samples are heterogeneous in their constitutive expression of some key ECM components such as collagenase and types I and III procollagen.³⁰ Moreover, the interaction of fibroblasts (harvested from site-matched papillary and reticular dermis) with keratinocytes and vascular endothelial cells reveals a significantly different release profile of growth

Figure 1. The schematic illustration of the production and the effect of proopiomelanocortin (POMC) and its derivatives on different cell types. POMC, synthesized in hypothalamus and pituitary gland, in response to corticotrophin-releasing hormone (CRH) secreted by hypothalamus, is released into circulation. POMC is also locally produced by keratinocytes and melanocytes. Differential enzymatic cleavage of POMC results in the production of different derivatives in various tissues. Human keratinocytes, melanocytes, dermal fibroblasts, follicular melanocytes, and follicular dermal papilla fibroblasts have the full potential to process POMC to α -melanocyte-stimulating hormone (α -MSH), adrenocorticotrophic hormone (ACTH), and β -endorphin. α -MSH binds to melanocortin 1 receptor (MC1R), increasing the eumelanin production by melanocytes, interleukin-10 (IL-10) expression by keratinocytes and monocytes, while reducing the synthesis of type I and III collagen by fibroblasts. The information for this figure was adapted from refs.⁷⁻¹³

factors and cytokines such as keratinocyte growth factor and granulocyte-macrophage colony-stimulating factor.^{31,32}

Several studies have shown the critical role of mesenchymal-epithelial cross talk in regulation of skin homeostasis and wound healing. Skin equivalents made of cocultured papillary fibroblasts with keratinocytes and implanted onto athymic mice showed a well-formed rete ridges; however, when reticular fibroblasts were utilized in coculture, there was a lack of rete ridge formation.³¹ The interaction of papillary fibroblasts with keratinocytes has also been shown to promote differentiation and basement membrane formation.³¹ Figure 2 illustrates the differences between papillary and reticular fibroblasts and their cross talk to keratinocytes in coculture systems. While papillary fibroblasts exhibit a higher migratory and proliferative capacity compared to reticular fibroblasts, the fibroblasts from deeper layers of skin express decreased levels of collagenase, but increased levels of TGF- β 1, collagen, versican, and α -smooth muscle actin (α -SMA) compared to papillary fibroblasts.³³ It has been suggested that these fibroblasts mimic HSc fibroblasts and may play a major role in HSc formation (Table 2).³³ Taken together, these findings in part explain the occurrence of HSc following deep dermal injuries versus superficial injuries.

Fibrocytes

Fibrocytes are unique subpopulation of leukocytes first described in 1994 by Richard Bucala.³⁴ While using

subcutaneously implanted wound chambers in mice to study acute cellular responses, the authors observed a rapid influx of many spindle-shaped, fibroblast-resembling adherent cells in addition to peripheral blood cells within 24 hours. Moreover, cultures of total leukocyte fraction of human peripheral blood, under conditions suitable for connective tissue cells, yielded the same population of proliferating, fibroblast-like cells, subsequently called fibrocytes. Further investigation showed fibrocytes expressing collagen, vimentin, and fibronectin (FN) as well as leukocyte common antigen CD45, pan-myeloid antigen (CD13), and the hematopoietic stem cell marker CD34.³⁴ Interestingly, the expression of MHC class II, costimulatory molecules CD80 and CD86, and the adhesion molecules CD11a, CD54 by fibrocytes makes them a potent antigen-presenting cell with ability to induce T-cell proliferation in the presence of specific antigen.³⁵

The role of fibrocytes in wound healing has been suggested by their rapid entry to the wound site. The signaling pathways involved in their recruitment and differentiation have been studied previously.³⁶ The authors showed that CD14⁺ cells from peripheral blood mononuclear cells (PBMCs) differentiated to fibrocytes in a process requiring contact with T cells, and TGF- β 1 promoted the fibrocyte differentiation and Col I expression by them within PBMC culture in a dose-dependent manner.³⁶ Moreover, the fibrocytes express chemokine receptor 7 (CCR7) and its ligand, secondary

Figure 2. Schematic representation of dermal fibroblast phenotypes and their interaction with keratinocytes in coculture system. Cultured fibroblasts harvested from papillary (within 300 μ m from the epidermis) and reticular (≥ 700 μ m) dermis reveal distinct morphologies, in addition to higher α -smooth muscle actin (α -SMA) expression of reticular fibroblasts when compared to their papillary counterparts. Coculture experiments of papillary and reticular fibroblasts with keratinocytes have shown the benefits of papillary fibroblasts for rete ridge and accelerated basement membrane formation. The information for this figure was adapted from refs.^{31,33}

Table 2. Comparison of morphologic and molecular characteristics of papillary, reticular, and HSc-derived fibroblasts

	Papillary fibroblasts	Reticular fibroblasts	HSc-derived fibroblasts
Cell size	↓	↑	↑
α -SMA + contraction of collagen gel	↓	↑	↑
Collagen	↓	↑	↑
Collagenase	↑	↓	↓
TGF- β 1	↓	↑	↑
Decorin	↑	↓	↓
Versican	↓	↑	↑

α -SMA, α -smooth muscle actin; HSc, hypertrophic scarring; TGF- β 1, transforming growth factor- β 1.

The information for this table was adapted from refs.^{28,30}

lymphoid-tissue chemokine, significantly induces the migration of the fibrocytes both in vitro and to the sites of cutaneous injury in vivo.³⁶ The unstimulated, cultured fibrocytes also express α -SMA and can contract collagen gels in vitro, similar to myofibroblasts, and pretreatment with TGF- β 1 increases both of these characteristics in cultured fibrocytes.³⁶ In a study on burn patients, Yang et al. showed that the percentage of type I collagen-positive fibrocytes developing in the cultures of PBMC derived from those patients was significantly higher than normal controls and this percentage had a positive correlation with the levels of serum TGF- β 1.³⁷ The

authors suggested that the increased level of TGF- β in serum of burn patients up-regulated the differentiation of the CD14⁺ cell population in PBMC into fibrocytes.³⁷ Later, leukocyte-specific protein 1 (LSP-1) was found to be expressed by fibrocytes, even more than lymphocytes, while it was absent in fibroblasts, suggesting it as a differentiating marker between fibrocytes and fibroblasts.³⁸ The collagen I⁺/LSP-1⁺ fibrocytes have been found to be present in hypertrophic and mature scar, but not in normal skin.³⁸ The higher number of fibrocytes in hypertrophic scar than mature scar was suggestive of their contribution to the development of HSc.³⁸ But significantly lower capacity of fibrocytes from burn patients or normal subjects to produce collagen than fibroblasts points to the indirect role of fibrocytes in the healing of burn wounds.³⁹ It has been shown that the medium conditioned by burn patient fibrocytes was able to increase cell proliferation, migration, and α -SMA expression by dermal fibroblasts.³⁹ The significantly higher levels of TGF- β 1 and connective tissue growth factor produced by burn patient fibrocytes might explain the regulatory effect of fibrocytes on fibroblasts.³⁹

Myofibroblasts

Myofibroblasts, first described in granulation tissue as an intermediate cell between fibroblasts and the smooth muscle cells, have been identified in healthy and pathological tissues.⁴⁰ Following tissue injury and disturbance of the mechano-protective architecture of the ECM, accompanied

by an overwhelming release of cytokines from damaged and inflammatory cells, a large panel of predominantly mesenchymal cells become activated, acquire myofibroblast phenotype, and drive tissue repair by secreting collagen and reorganizing the ECM.⁴¹ Although resident fibroblasts in different tissues are considered the most prominent precursor cells contributing to myofibroblast population, a variety of cells including hepatic stellate cells, fibrocytes, mesenchymal stem cells, smooth muscle cells, pericytes, and epithelial cells undergoing epithelial-mesenchymal transition can be the source of myofibroblasts.⁴¹ A comprehensive definition of the myofibroblast based on light microscopy features, immunophenotype, and ultrastructure has been given by Brian Eyden.⁴² Differentiated myofibroblasts exhibit both ultrastructural and functional features of smooth muscle cells, like contractile actin/myosin-containing stress fibers, and prominent rough endoplasmic reticulum seen in synthetically active fibroblasts.⁴¹ The most widely used marker for myofibroblast in clinical diagnostics and research is α -SMA.⁴¹ Although both myofibroblasts and smooth muscle cells are positive for α -SMA, the two cell types can be differentiated via immunostaining for desmin, smooth muscle myosin heavy chain, h-caldesmon, and smoothelin, which are positive in smooth muscle cells.⁴¹ Considering that normal tissue fibroblasts are negative for α -SMA and lack the contractile cytoskeleton, the term proto-myofibroblast has been introduced to describe α -SMA-negative fibroblast containing stress fibers, which appear in early granulation tissue.⁴¹ The stress fibers of proto-myofibroblasts are first composed of cytoplasmic actin and exert small traction forces.⁴³ The first collagen bundles deposited in the wound bed are laid down by proto-myofibroblasts.⁴¹ Consecutive differentiation of proto-myofibroblasts to α -SMA-positive myofibroblasts occurs in response to increased stress in ECM, growth factors like TGF- β 1, and specialized ECM proteins such as the FN splice variant ED-A FN.⁴³

In normal wound healing, the closure of the wound after reepithelialization is accompanied by significant decrease in cellularity of the granulation tissue, including myofibroblasts, which disappear mainly through apoptosis.⁴⁴ Other mechanisms have been described for disappearance of myofibroblasts, such as accelerated senescence and reversion to normal phenotype in some instances, like regression of liver fibrosis.^{45,46} Unlike normal wound healing where the appearance of myofibroblasts is limited temporally and spatially, persistence of myofibroblasts is a feature of fibroproliferative conditions like HSc, keloids, and burn wounds.⁴⁷ The underlying mechanisms involved in induction of myofibroblast apoptosis in normal wound healing or its persistence in pathological conditions have not been clarified.⁴⁸ However, since myofibroblasts are responsible for increased ECM stiffness and dependent on this function for differentiation, it has been suggested that myofibroblast differentiation and persistence in fibrotic scars are the result of feed-forward loops in myofibroblasts.^{47,49}

Role of Keratinocytes

The production of ECM in response to injury and its timely cessation is necessary for normal wound healing. An increasing body of evidence shows that the expression of

ECM components, including type I collagen by dermal cells is influenced by epidermal-mesenchymal interaction during wound healing. Clinical studies have shown that the risk of HSc increases when reepithelialization by keratinocytes is delayed.⁵⁰ On the other hand, a fine balance between ECM synthesis and its degradation by matrix metalloproteinases in remodeling phase of wound healing is a key factor in maintaining the structural integrity of the skin.⁵¹ In a keratinocyte/fibroblast coculture system, the collagenase expression in fibroblasts demonstrated a 10-fold increase compared to the fibroblasts in monoculture.⁵¹ Further experiments using keratinocyte-conditioned media (KCM) led to the identification of a keratinocyte-releasable factor with potent collagenase stimulatory effect on fibroblasts. The results revealed a releasable protein known as stratifin or 14-3-3 σ .⁵¹ Although keratinocytes express high levels of stratifin, both at mRNA and protein level, only the conditioned media derived from differentiated keratinocytes contain the releasable stratifin.⁵² It has been suggested that stratifin is one of the keratinocyte-derived anti-fibrogenic factors found in KCM, functioning as a wound healing stop signal(s) by modulating matrix metalloproteinase-1 expression in dermal fibroblasts.⁵¹ On the other hand, the expression of the type I procollagen in fibroblasts, cocultured with keratinocytes or treated with KCM, is significantly reduced both at mRNA and protein level.⁵⁰ These findings align with evidence showing early wound epithelialization is associated with reduced risk of HSc.

Macrophages and Hsc

The innate immune system is one of the first players involved in wound healing. Monocytes/macrophages as part of innate immune system not only play a critical role in generating and then resolving the inflammatory reaction to pathogens but also in maintenance of tissue homeostasis.⁵³ Since 1893 when macrophages were first described by Metchnikoff, the origin of tissue-resident macrophages and infiltrating macrophages during inflammation has been debated.⁵⁴ Recent evidence suggests that there are two lineage of tissue-resident macrophages: one originating from yolk sac earlier in embryonic life, populating different tissues and locating in close association with epithelial structures. These macrophages are independent of hematopoietic stem cells and Myb proto-oncogene protein, a member of the MYB (myeloblastosis) family of transcription factors, which is needed for the development of hematopoietic stem cells. The second lineage originates from hematopoietic stem cells and fetal monocytes which seed different tissues during embryogenesis.^{55,56} Although tissue-resident macrophages have self-renewal capacity, they can be replaced by monocyte-derived macrophages following inflammation or certain pathological conditions.⁵³ Both resident macrophages and bone marrow-derived macrophages demonstrate high plasticity in their function during an inflammatory reaction. They can be activated by different stimuli to exhibit a killing/inhibitory capacity (M1) at one end or heal/growth-promoting function (M2) at the other end of the functional spectrum.⁵³ While M1 macrophages are considered to be anti-fibrotic, M2 macrophages can increase pro-fibrotic activity via fibroblast proliferation and increased ECM production. A recent study by Zhu et al. found that depletion of M1 and M2 phenotypes

during the maximal abundance of M2 macrophages led to improved scar formation, collagen remodeling, and decreased infiltration of immune cells.⁵⁷ However, it is difficult to draw causal conclusions regarding M2 macrophage function, due to nonspecific inhibition during the subacute phase of wound healing.

Role of T Cells

Previous studies have shown that naïve T-helper cells (CD4⁺) adopt either Th1 or Th2 phenotype in response to certain cytokines and antigens.⁵⁸ Exposure to IL-12 promotes Th1 conversion and subsequent secretion of IL-2, interferon-gamma (IFN- γ), and tumor necrosis factor-beta; on the other hand, IL-4 exposure facilitates Th2 phenotype acquisition and secretion of IL-4, IL-5, IL-10, and IL-13.⁵⁸ After major traumas, such as burn injuries, there is a shift toward Th2 phenotype with increased IL-4 and IL-10 production in PBMC, but the production of IL-12 decreases.⁵⁸ Longitudinal studies in burn patients with HSc showed undetectable levels of IFN- γ in serum within 1 month of injury, in addition to an association between decreased IFN- γ circulating T cells (Th1) and levels of IL-12. However, in the early phases following burn injuries, the number of IL-4⁺ T cells (Th2), in addition to levels of IL-10 and TGF- β , were significantly higher in serum of burn patients who later developed HSc when compared with healthy volunteers and burn patients without HSc.⁵⁹ Further, increased number of CD3⁺ and CD4⁺ cells coexpressed with TGF- β , increased expression of IL-4 mRNA, and reduced IFN- γ mRNA in HSc tissues compared with normal skin and mature scar suggests an association between skin fibrosis and polarized Th2 systemic response to injury⁵⁹ (Figure 3). It has been shown that proportion of CD4⁺ T cells producing TGF- β are increased in the peripheral blood and HSc tissue of burn patients compared with healthy volunteers.⁶⁰ Remarkably, conditioned media, derived from purified T cells of burn patients, increased the proliferation and α -SMA expression of dermal fibroblasts in vitro, suggesting a crucial role for CD4⁺/TGF- β producing T cells in postburn HSc.⁶⁰ Additional Th subsets, namely Th17, Th22, Th9, and T follicular helper (Tfh) have been identified recently.⁶¹ The role of these subsets in HSc is not understood yet, but both Th17 and Th22 are important sources of IL-22, which has a significant role in tissue repair.⁶¹ IL-22 belongs to the IL-10 cytokine family and binds to a heterodimeric receptor consisting of IL-22R1 and IL-10R2.⁶² The ligand-binding subunit of the receptor, IL-22R1, is expressed by epithelial cells of the gastrointestinal tract and skin, through which IL-22 mediates the expression of genes involved in antimicrobial host defense by those cells.⁶² Additionally, IL-22 improves cell survival, induces epithelial cell proliferation, and restores epithelial barrier function.⁶² Consequently, IL-22 plays an important role in promoting resistance to extracellular pathogens, especially to gram-negative bacteria, by inducing the expression of antimicrobials, such as S100 proteins and defensins, and enhancing epithelial barrier function.⁶² As mentioned earlier, delayed epithelialization and bacterial colonization are important predictors of severe scarring.^{20,63} As a result, IL-22 may reduce the risk of HSc by promoting rapid epithelialization and minimizing bacterial colonization.

Transforming Growth Factor- β

TGF- β family, with three principal isoforms in mammals, plays a significant role in normal wound healing. Previous reports have demonstrated enhanced expression of TGF- β 1 mRNA in hypertrophic scar tissues and cultured fibroblasts in comparison to normal tissues and cells from the same patients.^{64,65} While TGF- β 1 and - β 2 are among the most important stimulators of collagen synthesis and inhibitors of its breakdown, TGF- β 3 reduces connective tissue deposition.²²

TGF- β 1, one of the critical growth factors involved in fibrosis, is synthesized as pro-TGF- β , consisting of covalently bonded TGF- β to latency-associated protein (LAP). Mature TGF- β , so-called small latent complex (SLC), is generated intracellularly by enzymatic cleavage of the covalent bond while TGF- β remains in association with LAP. SLC is the diffusible form of latent TGF- β secreted from a few cell types. In majority of cell types SLC covalently binds to latent TGF- β -binding protein within the secretory vesicles, generating large latent complex (LLC), which is then secreted from the cell. LLC is the reservoir of the latent TGF- β and binds to other ECM proteins through latent TGF- β -binding protein.⁴⁴ The latent form should be activated before TGF- β can bind to its cell surface receptor(s) and initiate the intracellular signaling pathway. Activation of latent TGF- β 1 involves dissociation of TGF- β 1 from SLC and/or ECM-bound LLC by various mechanisms, proteolytic cleavage being the most prominent one.⁶⁶

The SMAD signal-transduction pathway is a downstream mediator of TGF- β action. Signal transduction is initiated by TGF- β 1 binding to type I or II receptors, phosphorylating SMAD2/3 to bind SMAD4, forming a heterodimer complex that is translocated to the nucleus. SMAD2/3/4 complex subsequently induces targeted gene expressions, including type I and III collagens and FN.⁶⁷ Thus, TGF- β pathway has been a key target for the development of novel therapeutics. Recent in vitro studies demonstrated that loss-of-function microRNA-185 (miR-185) increased TGF- β 1 and type I collagen expression in HSc fibroblasts, whereas gain-of-function miR-185 in HSc fibroblasts decreased TGF- β and type I collagen. Future studies focusing on the in vivo aspects of these findings will provide insight on the effect of miR-185 on hypertrophic scars.⁶⁸

Integrins

Integrins are heterodimer, transmembrane receptors for cell-cell and cell-ECM adhesion, migration, and tissue organization. In mammals 18 α and 8 β subunits of integrin combine to produce 24 different heterodimers. The intracellular domain of integrins are part of the adhesion structures associated with cytoskeleton, and by binding to intracellular ligands they connect the extracellular receptor to signaling pathways, providing bidirectional transmission of mechanical stress to the cell and biochemical signals across the cell membrane.⁶⁹ The role of integrins in activation of latent TGF- β 1 was first reported in 1999.⁷⁰ One proposed mechanism for this function is facilitation of the enzymatic cleavage by simultaneous binding of integrins α v β 8 and α v β 3 to latent TGF- β 1 and proteinases and clustering of integrins with the TGF- β -RII.⁶⁶ The other suggested mechanism is direct activation of latent

Figure 3. A schematic representation of the T-cell phenotype, and cytokine profile in peripheral blood and hypertrophic scar (HSc) tissue of the burn patients. After burn injury, there is a shift toward Th2 phenotype and increase in interleukin-4 (IL-4), and transforming growth factor- β (TGF- β) in peripheral blood and HSc tissue, associated with decreased IL-12 and interferon (IFN)- γ . The information for this figure was adapted from refs.^{58,59}

TGF- β 1, independent from proteolysis, induced by integrin-mediated cell traction. In this model, mechanical forces cause conformational change in the LAP- β 1 structure, as part of LLC, resulting in activation of TGF- β 1.⁶⁶ The presence of actin cytoskeleton and specific integrins are the important prerequisites for the generation of mechanical force and/or resistance and transmission of the cellular traction forces to the LLC, as the reservoir of latent TGF- β 1 in a mechanically resistant ECM.⁶⁶

The exposed nature of integrins on the cell membrane and their sensitivity to blockade by ligand-mimetic antagonists have made integrins a potential therapeutic target.⁷¹

Decorin, Biglycan, and Versican

The interaction between ECM molecules and the local cells which produce them has significant role in tissue homeostasis and wound healing. One of the important ECM components are proteoglycans, glycosylated proteins with high water-binding ability, which stabilize the growth factors and cytokines within the ECM and contribute to the mechanical properties and three dimensional structure of the dermis.⁷² Decorin and biglycan are small nonaggregating proteoglycans involved in connective tissue organization through interaction with collagen fibrils.^{73,74} On the other hand, versican, the larger, aggregating proteoglycan, is associated with elastic fibers in the skin. Versican is considered to affect cell adhesion, migration, and proliferation. Although first described as inhibitor of cell adhesion to various ECM components, versican reportedly has adhesive properties as well.⁷⁵⁻⁷⁷ In an

investigation by Scott et al. on postburn hypertrophic scars, mature scars, and normal skin, the authors found that while decorin was abundant throughout the dermis in normal skin, it was absent or reduced in the nodular areas of the hypertrophic scars, in addition to being restricted to the deep dermis and a narrow zone under the epidermis. On the other hand, biglycan, versican, and TGF- β were undetectable in normal skin while readily seen in hypertrophic scars.⁷⁸ In normal skin decorin can bind TGF- β and possibly regulate its function by limiting the access of this cytokine to cell surface receptors. As a result, the lower expression of decorin might increase tissue response to TGF- β leading to HSc.⁷⁹

SUMMARY

Unraveling the factors involved in pathogenesis of HSc is the cornerstone of the successful prevention and/or treatment of this disturbing condition. For decades, clinically recognizable factors such as race, skin type, and depth of injury were found to be predictors of this pathologic scarring. The advancement in basic sciences has elucidated some of the underlying cellular and molecular mechanisms of HSc. Even though genetic determinants of HSc have not been identified, recent studies have found 70% and 100% allele frequencies of a loss-of-function SNP of the MCR1 gene among East/Southeast Asians and Native Americans, who are predisposed to HSc. Considering the effect of melanocortin signaling on reducing collagen expression by dermal fibroblasts, in response to TGF- β 1, one may

suggest that the loss-of-function SNP of MCR1 is one of the predisposing factors to HSc in certain races. The differential expression of ECM components, such as collagenase and types I and III procollagen, by fibroblasts, harvested from different layers of dermis, along with differences in their migratory and proliferative capacity in wound bed may partly explain the importance of depth of injury in HSc. Further, the cross talk between keratinocytes and fibroblasts, through keratinocyte releasable factor(s) such as stratifin, has a critical role in modulating collagenase and type I collagen expression in dermal fibroblasts. As it is also evident from clinical studies, early epithelialization reduces the risk of HSc. Macrophages play a significant role in inflammation and wound healing by acquiring killing/inhibitory (M1) or heal/growth-promoting (M2) capacities in response to various stimuli. Macrophages have the plasticity to switch between M1 and M2 and more studies are needed to define the role of these functional subtypes of macrophages in HSc. The role of CD4⁺ T lymphocytes, especially the Th2 subtype, in postburn HSc has been suggested by evidence of increased numbers of Th2 and its cytokine profile in peripheral blood of burn patients and subsequently in hypertrophic scar tissue.

One of the crucial factors involved in HSc is TGF- β 1, which is not only present as latent form in tissue reservoirs, but also produced during wound healing. The activation and downstream effect of TGF- β 1 has been the subject of many studies. Among various mechanisms, the role of integrin(s) in activation of TGF- β 1, through transmission of mechanical forces of cell/tissue traction and induced conformational change in TGF- β 1, may explain why the risk of HSc is higher in areas of skin under mechanical tension. Decorin is another factor affecting TGF- β activation by binding to it and restricting its availability to the cell surface receptors.

Despite the complexity of wound healing and the obstacles for in vitro and in vivo studies, the elucidation of some of the cellular and molecular mechanisms involved in the pathogenesis of abnormal scarring sets the stage for the development of more targeted therapies. In the future, development of topical formulations or dressings with sustainable release of anti-fibrosis agents will revolutionize acute wound care and prevent HSc.

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