The molecular mechanism of hypertrophic scar

Zhensen Zhu · Jie Ding · Heather A. Shankowsky ·
Edward E. Tredget

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Abstract Hypertrophic scar (HTS) is a dermal form of fibroproliferative disorder which often develops after thermal or traumatic injury to the deep regions of the skin and is characterized by excessive deposition and alterations in morphology of collagen and other extracellular matrix (ECM) proteins. HTS are cosmetically disfiguring and can cause functional problems that often recur despite surgical attempts to remove or improve the scars. In this review, the roles of various fibrotic and anti-fibrotic molecules are discussed in order to improve our understanding of the molecular mechanism of the pathogenesis of HTS. These molecules include growth factors, cytokines, ECM molecules, and proteolytic enzymes. By exploring the mechanisms of this form of dermal fibrosis, we seek to provide some insight into this form of dermal fibrosis that may allow clinicians to improve treatment and prevention in the future.

Keywords Hypertrophic scar · TGF- β · Cytokines · Decorin · Matrix metalloproteinases

Abbreviations

ALK 1	activin-receptor-like kinase 1
ALK 5	activin-receptor-like kinase 5
α -SMA	alpha smooth muscle actin
COL1	type I collagen
COL1A2	collagen type I α 2

COL3	type III collagen
COL4	type IV collagen
Co-SMAD	common mediator Smad
CTGF	connective tissue growth factor
ECM	extracellular matrix
ERK	extracellular signal-regulated kinase
HTS	hypertrophic scar
IGF-1	insulin-like growth factor-1
IL	interleukin
IFN	interferon
I-SMAD	inhibitory Smad
JNK	c-Jun N-terminal kinase
LSP-1	leukocyte specific protein-1
LTBP	latent TGF- β 1 binding protein
M6P/IGF II-R	Mannose 6-phosphate/insulin-like growth factor II receptors
MAPK	mitogen-activated protein kinase
MCP-1	monocyte chemotactic protein-1
MMP	matrix metalloproteinase
PKB	protein kinase B
R-Smad	receptor-regulated Smad
SDF-1	stromal cell-derived factor-1
SIP1	Smad interacting protein 1
SLRP	small leucine-rich proteoglycan
TGF	transforming growth factor
TGF- β R	TGF- β receptor
TIEG1	TGF- β inducible early gene 1
TIMP	tissue inhibitor of metalloproteinases

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Z. Zhu · J. Ding · H. A. Shankowsky · E. E. Tredget
Wound Healing Research Group, Department of Surgery,
University of Alberta, WMC 2D2.28, 8440-112 Street,
Edmonton AB, Canada T6G 2B7

E. E. Tredget (✉)
University of Alberta, 2D2.28 WMC, 8440-112 Street,
Edmonton AB, Canada T6G 2B7
e-mail: etredget@ualberta.ca

Introduction

Hypertrophic scars (HTS) are often caused by trauma and burn injury to the deep dermis and are itchy, raised, painful, rigid and disfiguring scars. Unlike keloids which progress beyond the original area of injury (Atiyeh et al. 2005), HTS remain within the boundary of the original injury. In many

cases, HTS occurs at the site of injury resulting in cosmetic disfigurement or when present in mobile regions of the skin, it can cause contractions that often result in limitation of joint mobility. These difficulties can lead to psychological and social issues for burn survivors (Engrav et al. 2007; Bombaro et al. 2003; De et al. 2009) (See Fig. 1). In this review, we have focused specifically on HTS and try to clarify the molecular mechanism of HTS, which would be helpful in developing new prevention and therapeutic treatments for people with HTS following injury.

Wound healing and the pathological features of HTS

Wound healing can be divided into four stages: hemostasis, inflammation, proliferation and tissue remodeling (Reinke and Sorg 2012). In these four stages, there are complicated interactions within a complex network of profibrotic and antifibrotic molecules, such as growth factors, proteolytic enzymes and extracellular matrix (ECM) proteins (Miller and Nanchahal 2005; Werner and Grose 2003). Each molecule plays its own part in the different phases of the wound healing process. As soon as the injury occurs, the hemostasis process begins and the bleeding is controlled by the aggregation of platelets at the site of injury. The subsequent formation of the fibrin clot helps stop the bleeding and provides a scaffold for the attachment and proliferation of the cells. Growth factors and cytokines are mainly secreted by the inflammatory cells and they contribute to the initiation of the proliferative phase of wound healing. Later, angiogenesis and collagen synthesis followed by tissue remodeling complete the stages of the wound healing process.

The delicate balance of deposition and degradation of ECM protein will be disrupted when either excessive production of collagen, proteoglycans and fibronectin by fibroblasts or deficient degradation and remodeling of ECM occurs (Tredget 1999). HTS occurs when the inflammatory response to injury is prolonged, leading to the pathological characteristics of HTS including increased vascularization, hypercellularity and excessive collagen deposition (Tredget

et al. 1997; Wang et al. 2011a, b; Armour et al. 2007). Our research group has also found decrease in the small leucine-rich proteoglycan (SLRP), decorin and increased transforming growth factor- β 1 (TGF- β 1) expression in HTS (Honardoust et al. 2012a, b).

Differences in cellular characteristics of normal dermal and HTS fibroblasts

Fibroblasts are the most common cells in connective tissue and are one of the key elements in wound healing. The main function of fibroblasts is to maintain the physical integrity of connective tissue, participate in wound closure as well as produce and remodel ECM (McDougall et al. 2006; Kwan et al. 2009). However, fibroblasts from HTS behave quite differently than normal fibroblasts. HTS tissue has greater amounts of fibroblasts that exhibit an altered phenotype than normal skin (Nedelec et al. 2001). HTS fibroblasts show higher expression of TGF- β 1 than normal fibroblasts (Scott et al. 1995). The increase or prolonged activity of TGF- β 1 leads to an overproduction and excess deposition of collagen by fibroblasts that often result in HTS formation (Shah et al. 1995). HTS fibroblasts have demonstrated reduced mRNA for collagenase as well as net reductions in the ability to digest soluble collagen as compared to their normal paired fibroblasts (Ghahary et al. 1996). HTS fibroblasts are also found to have a reduced ability to synthesize nitric oxide, an important mediator of growth factor signaling, which regulates wound healing and collagenase through its antiproliferative and antimicrobial effects (Wang et al. 1996).

HTS fibroblasts differentiate into myofibroblasts and can account for increased ECM synthesis and contraction of tissue. They are a particular phenotype which differ from fibroblasts by their expression of α -smooth muscle actin (α -SMA) (Nedelec et al. 2001). HTS myofibroblasts are less sensitive to apoptotic signals, coupled with their ability to produce more collagen and less collagenase than fibroblasts, which may play an

Fig. 1 Ten year old male with hypertrophic scar to the chest and flank 16 months following a burn injury

important role in HTS formation (Nedelec et al. 2000; Moulin et al. 2004).

Matrix metalloproteinase-1, 2, 9 (MMP-1, 2, 9) are involved in the formation of HTS regulated by tissue inhibitors of metalloproteinases (TIMPs)

The MMPs are a number of zinc-dependent proteinases, which are known for their critical role in the tissue remodeling process (Das et al. 2003; Yuan and Varga 2001). The most particular characteristic of MMPs is the function of the proteolytic cleavage of collagen and degradation of other elements of the ECM (Chakraborti et al. 2003). There are at least 23 types of MMPs. TIMPs are the specific proteins that regulate the function of MMPs and four specific types of TIMPs exist which block the activity of MMPs by binding to them in a 1:1 ratio (Stamenkovic 2003).

The over-expression of MMPs could result in an imbalance between ECM production and degradation, which could lead to chronic ulcers (Saito et al. 2001). Alternately, the alterations of MMPs and TIMPs expression could cause liver cirrhosis, the result of a reduction of collagen degradation and excessive accumulation of ECM (Lichtinghagen et al. 2001). As mentioned, TGF- β 1 acts as a potent inducer of the differentiation of myofibroblasts by stimulating the expression of α -SMA and it reduces the activity of MMPs by stimulating TIMPs synthesis in fibroblasts. In this way, the degradation process of ECM by MMP is abrogated. Meanwhile, the ECM deposition by fibroblasts is promoted by TGF- β 1. All of these features contribute to the formation of HTS (Desmoulière et al. 1993).

Many types of MMPs and TIMPs are considered to be related to HTS formation. Several studies showed that MMP-1 expression is decreased in HTS, an important transcriptional change in HTS, and its reversal could be a new therapeutic approach for the treatment of HTS (Reynolds 1996; Xie et al. 2008; Eto et al. 2012). Ghahary et al. showed that differentiated keratinocyte-releasable stratifin (14-3-3 sigma) could stimulate MMP-1 expression in dermal fibroblasts through c-fos and p38 mitogen-activated protein kinase (MAPK) pathway (Lam et al. 2005), which may control degradation of the major dermal ECM components and provide useful targets for clinical intervention of HTS formation (Ghahary et al. 2005). However, this stimulatory effect could be suppressed by insulin treatment (Lam et al. 2004). A subsequent study showed that the levels of MMP-2 and MMP-9 were up-regulated by the interaction between fibroblasts and keratinocytes, which may favor resolution of accumulated ECM components (Sawicki et al. 2005).

An early study showed that decreased expression of TIMP-1 with the subsequent increased secretion of MMPs caused delayed healing of chronic ulcers (Vaalamo et al. 1999). A study using athymic nude mice as an animal model

showed that MMP-9 was up-regulated in scarless wound healing (Manuel and Gawronska-Kozak 2006). Similar experiments demonstrated that the expressions of MMP-1 and MMP-9 were highly regulated in scarless fetal rat wounds (Dang et al. 2003). A human study of HTS, keloids, atrophic scars from different patients and different regions of the body suggested that MMP-9 played a leading role in scar-free healing (Tanriverdi-Akhisaroglu et al. 2009). A more recent study showed that by transfection of Smad interacting protein 1 (SIP1) to the HTS fibroblasts, MMP-1 expression was up-regulated while collagen type I α 2 (COL1A2) was decreased. As well, the knockdown of SIP1 in normal fibroblasts up-regulated COL1A2 levels induced by TGF- β 1. All these findings suggest that SIP1 could be a regulator of skin fibrosis and SIP1 depletion in HTS could result in the up-regulation of COL1A2 and the down-regulation of MMP-1, leading to excessive accumulation of ECM along with formation of HTS (Zhang et al. 2011b). Decreased levels of MMP-2, MMP-9 and increased levels of TIMP-1 were found in patients with HTS suggesting that the elevated systemic TIMP-1 concentration might contribute to tissue fibrosis, leading to HTS formation (Ulrich et al. 2003). A recent experiment compared TIMP-1 expression in normal skin and HTS, and the result exhibited relatively strong expression of TIMP-1 in HTS biopsies in contrast with very low levels of TIMP-1 in normal skin (Simon et al. 2012).

Depth of dermal injury is an important factor leading to HTS formation

The depth of injury is critical to HTS formation and of great clinical importance. The difference between superficial and deep injuries determines how these wounds heal and the severity of the scarring (Monstrey et al. 2008). Superficial wounds generally heal within 2 weeks without HTS formation and surgical treatment while deep wounds are prone to HTS and often require surgical interventions such as skin grafting and/or anti-scar procedures. In an experimental dermal scratch model done by the author's laboratory (Honardoust et al. 2012a; Dunkin et al. 2007), a specially designed and constructed jig was used to create progressively deeper wounds on a burn patient after informed consent. The wounds were along the lines of relaxed skin tension and were 6 cm long, 0 to 0.75 mm deep at one end (superficial wound) and 0.76 to 3 mm deep (deep wound) at the other end. The superficial wound of the wound scratch model healed with minimal scarring while the deep wound resulted in HTS that were red, raised, itchy scar confined to the site of injury. This result suggests there is a critical value of depth of the injury beyond which scar formation rather than tissue regeneration occurs (See Figs. 2 and 3). Thus, these

Fig. 2 Regeneration occurs in superficial wounds while scarring occurs in deeper wounds (From Kwan P, Hori K, Ding J, Tredget EE (2009) Scar and contracture: biological principles. *Hand Clin* 25(4):511–28; with permission)

findings suggest that deep dermal fibroblasts are responsible for HTS formation and maybe the source of the previously described characteristic HTS fibroblast.

Deep dermal fibroblasts resemble HTS fibroblasts

Superficial and deep dermal fibroblasts are derived from the papillary and reticular layers respectively and can be examined by using dermatomes to harvest different layers of the skin. Fibroblasts from the two layers exhibit heterogeneity, which may contribute to various outcomes of healing with different depths of injury (Sorrell and Caplan 2004). For example, injuries to the deep dermis where deep dermal fibroblasts are found most abundant often lead to the

development of HTS; whereas, superficial wounds injuring and activating a large number of superficial fibroblasts heal with little or no scar formation. Work published by our group proposed that deep dermal fibroblasts accounted for wound healing and HTS formation in deep thermal injuries where superficial fibroblasts were completely destroyed by the injury (Scott et al. 2000). Deep dermal fibroblasts produce more collagen (Ali-Bahar et al. 2004), proliferate slower (Feldman et al. 1993) and have less collagenase (Wang et al. 2008b) compared to superficial fibroblasts. Deep dermal fibroblasts also produced more α -SMA than superficial fibroblasts while it is all known that HTS fibroblasts are characterized by increased collagen synthesis and α -SMA expression. All of these provide evidence for the fibrotic characteristics of deep dermal fibroblasts, which

Fig. 3 Creation of deep and superficial scratch wounds and histological analysis of resulted scars. **a** Jig used to create the scratch wound model. **b** Wound created on the anterior thigh. **c** Scratch wound 70 days post-wounding. **d** Deep and superficial wound scar. **e** Deep wound scar tissue stained with hematoxylin and eosin staining (H&E). **f** Superficial wound scar tissue stained with H&E. Double-headed arrows in E and F indicate average thickness of epithelium. Arrowheads point to cells. Black arrows point to blood vessels. White arrows point to collagen. DW, deep wound; SW, superficial wound; DWS, deep wound scar; SWS, superficial wound scar (From Honardoust D, Varkey M, Marcoux Y, Shankowsky HA, Tredget EE (2012) Reduced decorin, fibromodulin, and transforming growth factor- β 3 in deep dermis leads to hypertrophic scarring. *J Burn Care Res* 33(2):218–27; with permission)

suggest a similar behavior between deep dermal fibroblasts and HTS fibroblasts. Studies conducted by our group showed that TGF- β and connective tissue growth factor (CTGF, also known as CCN2) (Wang et al. 2008b), two key profibrotic cytokines, were produced in greater quantities by deep dermal fibroblasts, which resembled a similar increase in the production of HTS fibroblasts (Wang et al. 2000). Thus, deep dermal fibroblasts resemble HTS fibroblasts and have similar biological functions distinctly different from superficial fibroblasts.

TGF- β isoforms are involved in HTS formation through the smad pathway

TGF- β is a secreted protein that exists in three distinct isoforms in mammals, TGF- β 1, TGF- β 2, and TGF- β 3 (Bock et al. 2005). Each isoform has a unique function in the wound healing process. TGF- β 1 and TGF- β 2 are secreted from degranulated platelets, as well as monocytes and macrophages, while TGF- β 3 is produced by keratinocytes. In normal tissue, the TGF- β isoforms exist as latent precursors where they are bound to latent TGF- β 1 binding proteins (LTBP) (Annes et al. 2003). Initially, it was thought that TGF- β contributed to wound healing by the stimulation of angiogenesis, proliferation of fibroblasts, differentiation of myofibroblasts and synthesis of collagen as well as deposition of ECM (McGee et al. 1989; Roberts et al. 1986). However, further experiments have shown that TGF- β plays an important role as a mediator in many diverse fibrotic disorders including pulmonary fibrosis, scleroderma (Broekelmann et al. 1991) and HTS (Ghahary et al. 1995b). In burn patients who developed HTS, the serum level of TGF- β was up-regulated locally and systemically (Tredget et al. 1998).

Shah et al. have shown that the two isoforms of TGF- β 1 and TGF- β 2 induce the formation of HTS (Shah et al. 1994) and the conclusion was consistent with the findings from Wang et al. (Wang et al. 2000). However, in adult rat incisional wounds treated with neutralizing antibodies of TGF- β 1 and TGF- β 2 reduced scarring occurred with the reduction of ECM deposition. Neutralization of either TGF- β 1 or TGF- β 2 did not have the same anti-scarring effect as neutralization of both TGF- β 1 and TGF- β 2. However, another experiment conducted by Shah et al. also suggested that TGF- β 3 inhibited scarring, indicating the TGF- β 3 might be the antagonist of the other two TGF- β isoforms (Shah et al. 1995). A more recent study by Leonard Lu et al. supported their findings. Rabbit ear wounds were treated intradermally with anti-TGF- β 1, 2, 3 antibodies at three different points and the result showed different effects in different periods of the wound healing process. Early treatment showed delayed wound healing while treatment in the middle or later stages of wound healing significantly reduced HTS. The impaired wound healing in the early stage

indicated that TGF- β isoforms were critical in the early phase of wound healing (Lu et al. 2005). TGF- β has multiple surface receptors, but TGF- β receptor I (TGF- β RI) and TGF- β receptor II (TGF- β RII) appear to be the predominant forms. TGF- β RI was found to be increased while TGF- β RII was decreased in HTS (Bock et al. 2005). Another experiment verified that truncated TGF- β RII could inhibit scar formation in rat wounds (Liu et al. 2005).

There are various types of signaling pathways for TGF- β 1. The most important one is the Smad pathway (See Fig. 4). Smads are intracellular regulatory signal transduction proteins that respond to activation of the TGF- β receptor complex. Smad proteins can be classified into three categories, the receptor-regulated Smads (R-SMADs), the common mediator Smads (Co-SMADs) and the inhibitory Smads (I-SMADs) (Kopp et al. 2005). Classically, intracellular signaling of TGF- β 1 is initiated after LTBP is dissociated from the complex. The activated TGF- β 1 is released and binds to TGF- β RII, which then activates the TGF- β RI. These two receptors are a ligand-dependent complex of heterodimeric transmembrane serine/threonine kinases. There are two isoforms of TGF- β RI that are involved in the signaling pathway known as activin-receptor-like kinase 1 and 5 (ALK 1 and ALK 5) (Pannu et al. 2007). The signaling pathway progresses when R-SMADs are phosphorylated by ALK 1 and ALK 5 respectively (ALK1 phosphorylates Smad 1, 5, 8 and ALK5 phosphorylates Smad 2, 3). The activated R-SMADs combine with the common mediator Smad 4 and then translocate into the nucleus where they function as transcription factors or participate in transcriptional control of other specific genes involved. I-SMADs (Smad 6 and Smad 7) antagonize the effects of the other two types of Smads, the R-SMADs and Co-SMADs by binding to TGF- β RI. In this way, phosphorylation of the R-SMADs and Smad 4 are inhibited. Thus, I-SMADs are considered to be the negative feedback regulators in the signaling pathway (Stopa et al. 2000). Decreased expression of Smad 7 in primary fibroblasts appears to decrease expression of type I collagen (COL1) and type III collagen (COL3) in a number of inflammatory disorders leading to fibrosis (Monteleone et al. 2004; Wang et al. 2002). Non-Smad signaling pathways such as MAPK, extracellular signal-regulated kinases (ERK) and the c-Jun N-terminal kinases (JNK) pathway have also been implicated with TGF- β signaling, but the exact mechanisms are not yet clear (Moustakas and Heldin 2005; Klass et al. 2009).

The abnormal intracellular signaling of TGF- β 1 is thought to initiate HTS by inducing the fibroblasts to excessively synthesize ECM and regulate CCN2, a downstream mediator of TGF- β 1 (Massagué 1998; Xu et al. 2004; Shi-wen et al. 2006; Klass et al. 2009). In chronic wound conditions, TGF- β 1 also has its influence, as the fibroblasts manifest an aberrant signaling pathway and decrease receptor expression, especially TGF- β RII (Kim et al. 2003). As the main component of TGF- β 1 signaling pathway,

overexpression of Smad proteins leads to the increased expression of COL1, COL3 and type IV collagen (COL4) (Cutroneo 2007). Importantly, Smad 3 knock out mice demonstrate faster wound healing, increased epithelialization and anti-scarring effects (Ashcroft et al. 1999; Bonniaud et al. 2004). Another study has shown that caveolin 1, one of the major coating proteins affecting TGF- β internalization and metabolism could inhibit TGF- β 1 by preventing Smad signaling in fibroblasts derived from HTS (Lee et al. 2007; Zhang et al. 2011a). A study conducted by our group recently showed that TGF- β inducible early gene 1 (TIEG1) knock out mice had a delay in wound closure due to impairment in wound contraction, granulation tissue formation, collagen synthesis and re-epithelialization, as well as increased Smad 7 in the wounds, which suggested a novel role of TIEG1 in dermal wound healing through the TGF- β /Smad signaling pathway (Hori et al. 2012). Insulin-like growth factor-1 (IGF-1) can also induce an increase in TGF- β mRNA and protein (Ghahary et al. 1998a). Mannose 6-phosphate/insulin-like growth factor II receptors (M6P/IGF II-R) were found to be involved in latent TGF- β 1 activation (Ghahary et al. 1999; Yang et al. 2000). As well, it was shown by our group that M6P/IGF II-R could facilitate the functional effect of IGF-1-induced latent TGF- β 1 on dermal fibroblasts (Ghahary et al. 2000) and a subsequent study has shown that latent TGF- β 1 may directly modulate fibroblast proliferation by a cell membrane dependent mechanism (Ghahary et al. 2002). In cell experiments, primary cultured dermal keratinocytes were genetically modified to express high levels of TGF- β 1 (Ghahary et al. 1998b). A later experiment co-cultured dermal fibroblasts with genetically modified keratinocytes, the result showed that latent and active TGF- β 1 could modulate ECM expression by overriding the effects of normal keratinocytes on the behavior of dermal fibroblasts (Bauer et al. 2002). Study using keratin 14-promoted TGF- β 1 transgenic mice showed excessive latent TGF- β 1 production in the epidermal layer of the skin where wounds exhibited a delay in re-epithelialization and early dermal fibrosis (Chan et al. 2002). Introduction of the latent TGF- β 1 gene into keratinocytes markedly increased the release and activation of TGF- β after burn injury (Yang et al. 2001). Thus, a central role of TGF- β 1 in the formation of HTS appears warranted.

Systemic response of Th1, Th2 and Th3 to HTS formation

The development of HTS involves a complicated interaction between inflammation and the immune response. Recent research suggests that it is not only the severity of inflammation, but the type of immune response links to the fibrotic

conditions (Wynn 2004). Wounds of thymectomized rats depleted of CD4+ lymphocytes showed a significant decrease in ultimate strength, resilience and toughness while wounds of animals depleted of CD8+ lymphocytes showed a significant increase in ultimate strength, resilience and toughness, suggesting that CD4+ lymphocytes play a very important role in wound healing (Davis et al. 2001). As well, it is known that CD4+ T lymphocytes predominate in HTS tissue with less CD8+ T lymphocytes (Castagnoli et al. 2002). Once activated by antigen presenting cells such as macrophages and dendritic cells, CD4+ T lymphocytes could differentiate into five subtypes of cells known as Th1, Th2, Th3, Th17 and T regulatory cells. Th1 and Th2 cells are the main subtypes defined in the murine model and each has a distinct cytokine profile (Mosmann and Coffman 1989). Th1 cells express interleukin (IL)-2, interferon (IFN)- γ and IL-12 while Th2 cells express IL-4, IL-5 and IL-10. Th1 cytokines contribute to increased collagenase activity and matrix remodeling, which is antifibrotic and in contrast, Th2 cytokines are known to be profibrotic (Doucet et al. 1998). According to the different effects caused by Th1 and Th2 cytokines, HTS fibroblasts are subject to a Th2 influence by presenting reduced collagenase activity and reduced NOS activity (Wang et al. 1996).

A burn mouse model showed diminished production of IL-2 and a shift in the Th2 phenotype with increased production of IL-4 and IL-10, indicating an inhibitory effect on Th1 function (O'Sullivan et al. 1995). Our group examined the Th1/Th2 cytokine ratio of 12 burn patients with HTS 4 weeks post-burn which showed a significant decrease in the Th1/Th2 ratio compared with 13 controls. The IL-4 levels in the patient group were significantly higher than controls and IFN- γ levels were significantly lower at 1 month post-burn, suggesting a role of the Th2 cytokine following burn injury (Kilani et al. 2005). Another study detected serum cytokine levels from 22 burn patients showed elevated Th2 levels of IL-4 and IL-10, and reduced Th1 levels of IFN- γ and IL-12. IL-4 mRNA levels in HTS tissue were increased, while IFN- γ mRNA levels were decreased compared to normal skin (Tredget et al. 2006).

Another study reported an increase in the frequency of CD4+/TGF- β -producing T cells in the peripheral blood and HTS of burn patients (Wang et al. 2007c). Medium derived from CD4+ T lymphocytes of burn patients was used to treat cultured dermal fibroblasts and results showed increased cell proliferation, collagen synthesis and α -SMA as well as a significant up-regulation of TGF- β compared to fibroblasts treated with medium derived from the CD4+ T lymphocytes of normal subjects. We suspect that these CD4+/TGF- β -producing T cells are identical to Th3 cells which are enhanced by TGF- β , IL-4 and IL-10. Thus, a polarized Th2 systemic immune response enhances the subsequent development of Th3 cells that are capable of

producing TGF- β , inhibits Th1 cytokine expression and finally contributes to HTS formation (See Fig. 5).

Fibrocytes, alternative precursors of myofibroblasts, play a pivotal role in HTS formation

Fibrocytes originate from the bone marrow and constitute 0.1 % to 0.5 % of peripheral blood cells (Quan et al. 2004). Fibrocytes have been reported to express surface markers CD34, CD45, collagen (Bucala et al. 1994) and leukocyte specific protein-1 (LSP-1) (Wang et al. 2007b). Peripheral blood fibrocytes can rapidly enter the site of injury along with inflammatory cells and participate in many aspects of wound healing, including production of ECM, antigen presentation, cytokine production, angiogenesis and wound closure (Chesney et al. 1997; Abe et al. 2001; Metz 2003). TGF- β 1 could induce the differentiation of fibrocytes into myofibroblasts through activating Smad2/3 and JNK MARK pathways (Hong et al. 2007) during wound healing (Mori et al. 2005).

A series of studies conducted by our group have established a role for fibrocytes in HTS formation. The peripheral blood of 18 burn patients was examined and the results showed increased fibrocytes compared to 12 controls (Yang et al. 2002). The number of fibrocytes was found higher in HTS than in mature scar tissue and a unique

surface marker protein, identified as LSP-1, was found in fibrocytes and in lymphocytes, but was undetectable in fibroblasts, allowing identification of these cells by double staining with antibodies to type I collagen and LSP-1 (Yang et al. 2005). LSP-1 is reported to be important in leukocyte chemotaxis. Excisional wounds in an LSP-1 deficient mouse model showed accelerated healing of full-thickness skin wounds with higher fibrocyte infiltration as well as up-regulation of TGF- β 1 (Wang et al. 2007a).

Chemokines contribute to HTS formation by recruiting monocytes into wound sites

Chemokines are small 8–10 kilodalton proteins that induce chemotaxis in responsive cells surrounding the site of injury. They can be divided into four types depending on the spacing and location two cysteine residues in the molecules and include CC, CXC, C and CX₃C subfamilies (Fernandez and Lolis 2002).

SDF-1, also known as CXCL12, belongs to CXC group. SDF-1 is similarly expressed in humans, swine and rat skin and is produced by pericytes, endothelial cells and fibroblasts (Mirshahi et al. 2000). CXCR4 is a CXC chemokine receptor and it exclusively binds to SDF-1, which is unique among receptors because most chemokines have more than one receptor and most receptors have more than one ligand

Fig. 4 The TGF- β 1 Smad signaling pathway contributes to HTS. TGF- β RI is activated after TGF- β 1 binds to TGF- β RII. R-SMADs are then phosphorylated by ALK1 and ALK 5, two isoforms of TGF- β RI. The activated R-SMADs bind with Smad 4 and then translocate into nucleus and act as transcription factors. I-SMADs antagonize the effects of the R-SMADs and Co-SMADs

Fig. 5 Hypothetical diagram of the role of Th1/Th2/Th3 cells in stimulating bone marrow stem cells to healing wounds. (From Armour A, Scott PG, Tredget EE (2007) Cellular and molecular pathology of HTS: basis for treatment. Wound Repair Regen 15:S6-17; with permission)

(Choi and An 2011). SDF-1/CXCR4 signaling pathway mediates the migration of hematopoietic cells from fetal liver to bone marrow (Zou et al. 1998), and could also stimulate angiogenesis by recruiting progenitor cells (Nagasawa 2001). Early studies focused on the functions of SDF-1/CXCR4 signaling in the regulation of stem/progenitor cell trafficking, especially tumor cells metastasis and tumor vascularization (Balkwill 2004). However, recent studies have suggested that SDF-1/CXCR4 signaling participates in the pathogenesis of lung injury and fibrosis (Xu et al. 2007). An experiment using skin from burn patients showed an increased expression of SDF-1 in human burn blister fluid and improved skin recovery after blocking the SDF-1/CXCR4 pathway (Avniel et al. 2006). A more recent study from our research group showed up-regulated SDF-1/CXCR4 signaling in burn patients with increased SDF-1 levels in HTS and serum. Our study showed that SDF-1/CXCR4 signaling was important in the pathogenesis of HTS by stimulating the migration of activated CD14⁺ CXCR4⁺ cells to migrate to the injured tissue. These migrating cells may differentiate into fibrocytes and myofibroblasts and contribute to the pathogenesis of HTS (Ding et al. 2011; Wang et al. 2007c).

Another chemokine linked to the formation of HTS is monocyte chemoattractant protein-1 (MCP-1). It belongs to the CC chemokine subfamily and has two receptors, CCR2 and CCR4 (Craig and Loberg 2006). Being a major chemoattractant, it is secreted by macrophages, endothelial cells and fibroblasts. MCP-1 recruits monocytes and dendritic cells to the inflammatory sites (Hasegawa and Sato 2008; Xu et al. 1996). A previous study showed that MCP-1 could stimulate fibroblast collagen production in the lung through an up-regulation of endogenous TGF- β (Gharraee-Kermani et al. 1996). A later experiment investigated the role of MCP-1

in fibrosis using MCP-1 knockout mice. Results showed that fibrosis was diminished in MCP-1 knockout mice compared to the bleomycin-induced fibrosis control group (Ferreira et al. 2006). Enhanced release of MCP-1 by keloid CD14⁺ cells stimulated fibroblast proliferation through the protein kinase B (PKB) signaling pathway and might trigger keloid development (Liao et al. 2010). A study from our research group demonstrated a significant increase of MCP-1 expression in fibroblasts from HTS compared to normal fibroblasts further suggesting a dominant role for MCP-1 in fibrotic diseases (Wang et al. 2011b).

Antifibrotic molecules, decorin and IFN- α 2b inhibit HTS formation by inhibiting TGF- β and regulating fibroblasts

Recently, more research focuses on SLRPs, a small component of ECM molecules, which contain several repeated leucine-rich regions and cysteine residues. The major SLRPs are decorin, biglycan, lumican, and fibromodulin (Sidgwick and Bayat 2012). The most common SLRPs studied is decorin that is abundantly expressed in the dermis and in connective tissue (Honardoust et al. 2011). As well, it appears to promote collagen fibrillogenesis, cell differentiation and inhibits the bioactivity of growth factors such as TGF- β (Schönherr and Hausser 2000). We have reported different levels of decorin in burn wounds with suppressed expression for the first 12 months, then significantly higher expression after one to two years, decreasing to resemble the expression seen in normal skin after 3 years (Sayani et al. 2000). Decorin is considered to be an inhibitor of TGF- β . Animal studies using bleomycin-injected mice treated with adenovirus vector-derived decorin showed that decorin

significantly reduced the fibrosis caused by bleomycin by blocking the fibrotic effect of TGF- β (Kolb et al. 2001). Wang et al. previously reported that deep dermal fibroblasts might contribute to HTS formation because of a decrease in decorin production (Wang et al. 2008a). Recently, decorin was used to treat superficial and deep dermal fibroblasts where increased cell apoptosis was seen in superficial dermal fibroblasts compared to deep dermal fibroblasts, suggesting decorin-induced apoptosis might play an anti-fibrotic role (Honardous et al. 2012a, b). Further study is needed to clarify the interaction between SLRPs and TGF- β 1 in superficial and deep wounds to demonstrate how the decreased expression of decorin and TGF- β 1 in deep dermal fibroblasts contributes to the formation of HTS (Honardous et al. 2012a).

IFN, known as a Th1 cytokine, could be divided into three subtypes, type 1 IFN (including IFN- α and IFN- β), type 2 IFN (IFN- γ) and type 3 IFN (IFN- λ) (Ladak and Tredget 2009). IFN- α is produced by leukocytes and fibroblasts, while IFN- γ is produced by T lymphocytes such as activated T cells (Sarkhosh et al. 2003). IFN- α and IFN- γ could decrease cell proliferation and collagen synthesis in normal and HTS fibroblasts in vitro (Tredget et al. 1997). IFN- α 2b is an antiviral drug originally used for viral infections and some forms of cancer and has recently been shown to increase collagenase mRNA levels and activity and reduce the activity of TIMP-1 (Ghahary et al. 1995a), thereby decreasing HTS formation. Further studies were carried out in the authors' laboratory focusing on IFN- α 2b because of the antifibrotic effects it exhibited. IFN- α 2b exposure to matched pairs of human HTS and normal fibroblasts could inhibit wound contraction by decreasing fibroblast-populated collagen lattices (Nedelec et al. 1995). Patients with severe HTS treated with IFN- α 2b showed a significant improvement in scar quality and volume during the therapy with a significant decrease in serum TGF- β levels (Tredget et al. 1998). Treating HTS and normal fibroblasts with IFN- α 2b or IFN- γ demonstrated that TGF- β protein production was antagonized in part by the down-regulation of TGF- β 1 mRNA levels (Tredget et al. 2000). Additionally, IFN- α 2b treatment decreased angiogenesis in HTS tissue (Wang et al. 2008a). In a double blind placebo controlled trial in 21 burn patients conducted by our group, the administration of IFN- α 2b to burn patients down-regulated SDF-1/CXCR4 signaling in HTS tissues limiting the formation of HTS (Ding et al. 2011), which supported the contention that IFN- α 2b is an antifibrotic protein inhibiting HTS formation.

We have reviewed the important factors that impact HTS formation. Increasing evidence indicates that HTS develop after multifactorial interactions of TGF- β 1, Th1/2/3 cytokines, decorin and MMPs acting in different phases of the wound healing process.

The treatment of HTS

Current treatment of HTS remains time-consuming, expensive and with few consistently successful approaches. One of the difficulties is that the outcome of HTS formation varies between patients, scar location and between conservative interventions. So it is extremely difficult for surgeons to predict which scars need surgical excision or which scars might resolve over time (Tredget et al. 1997). There are however a variety of therapeutic options available to treat HTS, including surgical excision and non-surgical treatment.

Surgical therapy for HTS

Surgical excision is common management when used in combination with steroids and/or silicone gel sheeting. However, surgical excision of HTS without adjuvant therapy is associated with a high rate of recurrence, ranging from 50 % to 80 % (Darzi et al. 1992). HTS resulting from excessive tension or wound complications can be treated effectively with surgery options including intramarginal excision, skin grafts, local flaps and free flaps combined with surgical taping and silicone gel sheeting (Mustoe et al. 2002). But these technique are not suitable for immature scar.

There are two major kind of lasers, ablative nonselective lasers such as CO₂ laser and nonablative selective lasers such as pulse-dye laser. Ablative lasers might carry a higher risk while nonablative lasers have the advantage of improving scars without incision or wounding. The flash lamp-pumped pulsed dye laser is extensively used to refine scars by causing direct destruction of the blood vessels and an indirect effect on the surrounding collagen, which result in collagen modeling and wound contraction (Lee et al. 2005).

Non-surgical therapy for HTS

Non-surgical management of HTS includes the use of pressure garments, intralesional corticosteroid administration, silicone gel sheets and so on. Pressure garment are commonly used and are supplied by several companies based on individual patient measurements (Macintyre and Baird 2006). Pressure therapy should be applied 24 h a day until the scar is mature. The optimum pressure for effective treatment is still unknown, but the pressures applied should exceed capillary pressure and recommend that pressure be maintained between 24 and 30 mmHg (Mustoe et al. 2002). The possible mechanism of pressure garment may relate to reduced fibroblast proliferation, decreased collagen synthesis, increased myofibroblasts apoptosis (Armour et al. 2007; Anzarut et al. 2009).

Intralesional corticosteroid administration is also widely used to alleviate HTS. Triamcinolone acetonide is the most commonly used corticosteroid. The administration should be

confined to the dermal region of the scar with 10 to 40 mg/ml at 2- to 6-weeks interval (Atiyeh 2007). Silicone gel is a cross-linked polymer of dimethylsiloxane, which has been used for treatment of immature scar. Applying silicone gel sheet topically is a noninvasive and relatively safe treatment since it may decrease the volume of HTS (Ahn et al. 1989). It has been shown that silicone gel sheets may accelerate scar maturation and improve pigmentation, vascularity, pliability, pain and itchiness associated with HTS (Momeni et al. 2009). Thus, prophylactic use of topical silicone gel following scar revision surgery may prevent the development of recurrent HTS (Gold et al. 2001).

Future perspective

Despite the complexity of HTS, significant accomplishments have been made to establish a number of wound healing and dermal fibrotic animal models. Recently the author's laboratory has transplanted human skin onto the backs of nude mice creating a human HTS-like nude mouse model. An increase infiltration of fibrocytes and macrophages has been observed, which suggest that they may contribute to HTS development (Wang et al. 2011a). Meanwhile, more research findings have suggested a critical role of macrophages in fibrotic diseases (Lucas et al. 2010; Murray et al. 2011). Thus, our future perspective will be focusing on the roles of the macrophages and different types of macrophages in the the development of HTS using the nude mouse model, which will assist clinicians in the development of novel forms of treatment to prevent and treat HTS and other fibrotic disorders.

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