Summary. Hypertrophic scars (HTS) are predominant diseases after burn and trauma, which cause severe physiological and psychological problems. HTS have been researched for decades, and our knowledge about the mechanisms of HTS formation process has been increasing. However, the effects of currently available prevention and treatment strategies are limited. In this review, we summarize currently known mechanisms and recent studies of HTS, including extracellular matrix, matrix metalloproteinases, fibroblasts, myofibroblasts and their contraction ability, keratinocytes, growth factors, inflammatory and immune response, and stem cell treatment, hoping for a better understanding of HTS generation, development and effective translation to treatment strategies.

Key words: Hypertrophic scars, Fibroblasts, Growth factors, Mechanotransduction, Extracellular matrix

Introduction

The skin wound healing is one of the most important physiological processes in life, which restores the integrity of skin, protects patients from infection and body fluid loss. However, a scarless wound healing is unachievable for now. Except for the superficial wounds, the injury to deep layers of the dermis ends up with scar formation, which is subtle and hardly visible. But when the balances are broken, the injury develops into non-healing wounds or pathological scars including hypertrophic scars (HTS) and keloid. HTS are often occurred after surgery, burn, trauma and other injuries of skin, which are raised, red, painful, itchy, contractile, and different from keloid within the site of injury (Berman et al., 2017). When crossing joints, HTS cause severe restriction of joint movement. In addition to aforementioned aesthetic problems, HTS lead to great psychological and physiological burden (Chiang et al., 2016). The major risk factors of HTS include deep dermal injury, high tension, young age and so on (Butzelaar et al., 2016a).

The normal wound healing process contains three periods, namely, inflammation, proliferation and remodeling period. After injury, the inflammatory reaction occurs immediately. It begins with the clotting cascade, during which process platelets and monocytes act as the starters of inflammation (Imhof et al., 2016). Then neutrophils and monocytes appear in the wound region, and monocytes then differentiate into macrophages, which play a critical role in the whole process of wound healing. Other immune cells are also recruited into the wound site. The proliferation period is characterized by the migration of keratinocytes, the differentiation of epithelial stem cells, the ingrowth of new blood vessels and the accumulation of extracellular matrix (ECM) by fibroblasts and myofibroblasts. The myofibroblasts could also provide the strength for wound contraction (Zhu et al., 2016a). The last stage, remodeling, lasts for a year or more. In this stage, ECM is remodeled from a mainly type III collagen to one predominantly composed of type I collagen, and this
the process is mainly controlled by fibroblasts and macrophages with the assistance of MMPs. If any mistake happens during the three processes, the injury ends up with non-healing wound or pathological scars including keloid and HTS. HTS are characterized by excessive collagen deposition, increased proliferation of fibroblasts and myofibroblasts, the disorder of growth factors, increased vessel density, and abnormal inflammation and immunity. We will focus on those characteristics and will review recent advances of mechanism and its translation to treatment strategies.

**ECM changes in HTS, and matrix metalloproteinases participate in the degradation**

ECM, which consists of collagens, fibronectins, laminins, elastins, proteoglycans, hyaluronic acid (HA) and glycoproteins such as integrins, was thought to only provide the structural integrity. However, recent research indicates the important functions of ECM in providing a template for cells, binding to integrins and other receptors, signal transduction and providing growth factors which can be released under wounded situation (Xue and Jackson, 2015). Scars are composed of the same ECM molecules as the tissue they replace, but the ratios in scar tissue are different from normal tissue. HTS are characterized by excessive deposition and alteration in morphology of collagens and other ECM proteins (Xue and Jackson, 2015). The levels of collagen I and III, fibronectins, and laminins are all increased in HTS tissue. On the contrary, HA and decorin are decreased, and the expression and localization of fibrillin and elastin fibers in the dermis are altered in HTS compared with normal skin and non-hypertrophic scars (NTS) (Profyris et al., 2012).

The changes of ECM provide potential therapeutic strategies for HTS. Decorin, as a natural neutralizer of transform growth factor beta (TGF-β) 1, inhibited fibroblast proliferation, decreased TGF-β1 production and collagen synthesis in HTS (Xue and Jackson, 2015). Reduced decorin, fibromodulin, and TGF-β3 in deep dermis lead to HTS, and myostatin-null mice exhibited delayed skin wound healing through the blockade of TGF-β signaling by decorin (Zhang et al., 2012; Honardoust et al., 2012a). Blocking miR-181b, which was increased in deep dermal fibroblasts, could reverse the downregulation of decorin and the transdifferentiation of myofibroblasts in HTS, which indicates a potential treatment for HTS (Kwan et al., 2015). Other components of ECM such as integrins and integrin-linked kinase (ILK) also participate in HTS formation, which will be discussed later.

The matrix metalloproteinases (MMPs) are thought to be primarily involved in the ECM degradation. They are regulated by tissue inhibitors of matrix metalloproteinases (TIMPs) in a ratio of 1:1. Fibrillar collagens can be degraded by specific collagenses, especially MMP-1, MMP-8, and MMP-13. After degradation of ECM by collagenses, MMP-2 and MMP-9 are responsible for the degradation of the remaining product such as gelatin (Xue and Jackson, 2015). The imbalance of ECM deposition and MMPs expression could cause HTS (Dang et al., 2003). Decreased levels of MMP-1, MMP-2, MMP-9 and increased level of TIMP-1 are present in HTS and contribute to tissue fibrosis, leading to excessive scars (Xue and Jackson, 2015). Knockdown of TIMP-1 with siRNA in keloid fibroblasts resulted in reduced collagen-I deposition, which was mainly a result of increased MMP2 activity (Aoki et al., 2014). Another study using transgene of TIMP-1 in human fibroblasts resulted in increased proliferation of fibroblasts and expression of TGF-β1 and α-SMA (Sa et al., 2015), suggesting that TIMP1 may regulate cell activation, but its role in HTS remains to be further investigated. Several researchers found it is hopeful to suppress HTS through up-regulation of MMP1 (Lee et al., 2015). Recent studies demonstrate that MMPs not only participate in ECM degradation, but also are involved in processes of immunity, cell migration, and angiogenesis (Rohani and Parks, 2015). Macrophages are the main source of MMPs as well as TIMPs. Recently, Rohani et al. (2015) found that knockout of MMP-10 resulted in increased collagen deposition and skin stiffness, accompanied with reduced expression of MMP-8 and MMP-10. They proved that M2 macrophages are the main cells participating in MMP-10 dependent collagen-degradation activity through the secretion of other MMPs (Rohani et al., 2015).

**Main effectors of skin fibrosis and contraction: fibroblasts and myofibroblasts**

Fibroblasts and myofibroblasts are thought to be the main fibrogenesis effectors (Hinz, 2016). Myofibroblasts are differentiated from fibroblasts under the stimulation of TGF-β1 and mechanical force through several integrins (Van De Water et al., 2013), in which process myocardin-related transcription factors play an important role (Crider et al., 2011). HTS tissue contains a greater number of fibroblasts and myofibroblasts than normal skin and NTS. HTS fibroblasts exhibit increased collagen I synthesis, decreased collagenase production and subsequently reduced ability to digest soluble collagen compared to normal fibroblasts. Myofibroblasts provide the necessary contractile force to close the deep wounds. However, in HTS tissue, myofibroblasts fail to undergo apoptosis or deactivation, which are the routine procedures in normal wound healing. On the contrary, their continuing activation results in excessive ECM deposition and contraction of scar (Chiang et al., 2016; Huchtenreuther and Leask, 2016). p53, which induces cell cycle arrest and apoptotic cell death, has a higher incidence of mutations (exons 5-8) in both keloid and HTS fibroblasts (Xue and Jackson, 2015). Besides local fibroblasts, myofibroblasts can also derive from pericyte, fibrocytes, adipocyte and epithelial-mesenchymal transition derived cells (Van De Water et al., 2013; Lian and Li, 2016). The role of the myofibroblasts from...
different sources remains unclear, but there are several studies indicating the different functions of the myofibroblasts with different origins. In skin fibrosis model, researchers found that SOX2-expression progenitor cells-derived myofibroblasts were predominant in skin fibrosis, while in normal wound healing, SOX-2 expression progenitor cells were not the main source, and connective tissue growth factor (CTGF/CCN2) was necessary for the recruitment of these progenitor cells (Liu et al., 2014a; Tsang and Leask, 2015). More works are needed to further understand the origin of myofibroblasts and its impact on scar formation. Interestingly, there is a study demonstrating that myofibroblasts may contribute to, but are not necessary for wound contraction. After knockout of α-SMA (ACTA2), which is thought to be critical for the trans-differentiation and function of myofibroblasts, the author found that wound contraction was retarded but did not disappear in knockout mice, and the collagen deposition was reduced compared with control group (Ibrahim et al., 2015). However, another study partly explained the outcome by finding that after α-SMA knockout, smooth muscle γ-actin and skeletal muscle α-actin may play compensatory roles to maintain the normal function and morphology of myofibroblasts (Tomasek et al., 2013).

Injury depth partly determines whether HTS are formed. Efforts to find the reason never stopped. Researchers compared the fibroblasts from the deeper layers with fibroblasts from superficial layers and found that the former express more TGF-β1, CTGF, α-SMA and collagens but less decorin, fibromodulin and TGF-β3 (Wang et al., 2008; Honardoust et al., 2012a). Meanwhile, they found that deep dermal fibroblasts showed lower migration, and less apoptosis when treated with decorin (Honardoust et al., 2012b). Recently, two studies provided exciting finds. Driskell et al. (2013) demonstrated two distinct fibroblasts from the upper and lower dermis by using transplantation assays and lineage tracing in mice. One forms the dermal papilla that regulates hair growth and participates in re-epithelialization, while the other forms the reticular fibroblasts and is in charge of synthesizing the bulk of fibrillar ECM (Driskell et al., 2013). This is quite attractive considering the truth that deep partial thickness burn wounds exhibit delayed re-epithelialization and high risk of HTS formation which contain less hair follicle. Then, they found the different effects of TGF-β2 and Hedgehog (Hh) on papillary or reticular fibroblasts following Wnt/β-catenin activation (Lichtenberger et al., 2016). Research from another group found that fibroblasts derived from engrailed-1 (En-1) positive cells are responsible for the bulk of connective tissue deposition and CD26/DPP4 is a surface marker of this lineage (Rinkevich et al., 2015). Lineages specific depletion of fibroblasts revealed scarless wound healing without tensile strength reduction. The inhibitor of CD26 in the wound bed resulted in diminished cutaneous scar formation. The aforementioned researches also support the finding about CD26 (Driskell et al., 2013; Lichtenberger et al., 2016). More attention should be focused on the role of different lineages in HTS, expecting for cell-specific understanding and treatment of HTS.

**High tension of skin and mechanotransduction**

It is well known that wounds in the area of skin with high tension have a high risk of HTS formation (Kuang et al., 2015). The related research found that fibroblasts derived from the different parts of human skin showed different reactivity to mechanical stimulation, as fibroblasts derived from scapular upper skin were observed to proliferate at a higher rate compared with fibroblasts divided from medial side of upper arm (Kuang et al., 2015). These results drive researchers to work out the relationship between mechanical force and HTS. Several pathways including calcium dependent iron channel, focal adhesion and integrin participate in the process of mechanical transduction (Fig. 1) (Chiang et al., 2016). Mechanical signaling is an important factor which influences collagen deposition, myofibroblast differentiation and cytokine secretion. Accompanied by larger focal adhesions, myofibroblasts gain more α-SMA, and this change of focal adhesions is related to Rho GTPase-dependent network (Van De Water et al., 2013). Integrins are thought to be the main focal adhesion mechanoreceptor in cells. When there is enough mechanical force, myofibroblasts cytoskeleton is in charge of synthesizing the bulk of fibrillar ECM (Driskell et al., 2013). This is quite attractive considering the truth that deep partial thickness burn wounds exhibit delayed re-epithelialization and high risk of HTS formation which contain less hair follicle. Then, they found the different effects of TGF-β2 and Hedgehog (Hh) on papillary or reticular fibroblasts following Wnt/β-catenin activation (Lichtenberger et al., 2016). Research from another group found that fibroblasts derived from engrailed-1 (En-1) positive cells are responsible for the bulk of connective tissue deposition and CD26/DPP4 is a surface marker of this lineage (Rinkevich et al., 2015). Lineages specific depletion of fibroblasts revealed scarless wound healing without tensile strength reduction. The inhibitor of CD26 in the wound bed resulted in diminished cutaneous scar formation. The
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![Diagram of immune response and HTS formation](image)

Fig. 1. Immune response and HTS formation. In HTS, T cells show a Th2 predominant phenotype, which is characterized by the secretion of IL-4, IL-10 and IL-13. These cytokines are critical to the polarization of macrophages to a M2 phenotype, which also proved to be the major phenotype of macrophages in HTS. Th17 participates in the recruitment and activation of neutrophils and monocytes, and Tregs modulate immune response through IL-10 and TGF-β. Their imbalance could lead to chronic inflammation and fibrosis. However, their effects on HTS have not been investigated.

Keratinocytes participate in HTS formation in several ways

Keratinocytes are the most dominant cells in epidermis. Usually, they move from basal layer towards the surface, undergoing the process of differentiation at the same time. Eventually, keratinocytes arrive at the cornified envelope and become part of it (Pastar et al., 2014). When injured, they proliferate and migrate into the wound, playing a key role in re-epithelization. A recent study found that in HTS tissue, epidermal thickness is increased compared with normal skin. Besides, keratinocytes derived from HTS showed different markers and increased proliferation compared with which from normal skin. The author demonstrated that this difference of keratinocytes is caused by absence of basement membrane in HTS (Yang et al., 2016). Another study revealed keratinocytes in HTS underwent excessive differentiation and participated in the formation of HTS through their interaction with fibroblasts (Li et al., 2016a). Actually, keratinocytes could regulate a series of genes of collagen production when co-cultured with fibroblasts in a transwell model, including the upregulation of MMP-1, MMP3 and the downregulation of CTGF, collagen I, collagen III, fibronectin, PAI-1, TIMP-2, TIMP-3 and α-SMA. Keratinocytes can also affect migration, proliferation and apoptosis of fibroblasts (Koskela et al., 2010). TIMP-1 is secreted by keratinocytes. The latest study demonstrated that increased secretion of TIMP-1 by human HTS

synthesis was reduced, making FAK a potential effective therapeutic approach for HTS (Chen et al., 2014a). Besides, mechanical force induces less apoptosis of fibroblasts and stronger inflammation, promoting the HTS process (Aarabi et al., 2007; Wong et al., 2012; Cremers et al., 2015). Considering the relationship between mechanical force and HTS formation, two clinical trials were performed with a tension-reducing device (Lim et al., 2014; Longaker et al., 2014). In these trials, patients after surgery were treated with the Advanced Scar Therapy device, which could decrease the mechanical force on surgical incisions. After 6 or 12 mouth, the scar formation was significantly reduced compared with control group. This device provides a novel therapy for scar prevention.

Aside from these clinical trials, keratinocytes are also crucial in the HTS process. They play a key role in the formation of the cornified envelope and become part of it (Pastar et al., 2014). When injured, they proliferate and migrate into the wound, playing a key role in re-epithelization. A recent study found that in HTS tissue, epidermal thickness is increased compared with normal skin. Besides, keratinocytes derived from HTS showed different markers and increased proliferation compared with which from normal skin. The author demonstrated that this difference of keratinocytes is caused by absence of basement membrane in HTS (Yang et al., 2016). Another study revealed keratinocytes in HTS underwent excessive differentiation and participated in the formation of HTS through their interaction with fibroblasts (Li et al., 2016a). Actually, keratinocytes could regulate a series of genes of collagen production when co-cultured with fibroblasts in a transwell model, including the upregulation of MMP-1, MMP3 and the downregulation of CTGF, collagen I, collagen III, fibronectin, PAI-1, TIMP-2, TIMP-3 and α-SMA. Keratinocytes can also affect migration, proliferation and apoptosis of fibroblasts (Koskela et al., 2010). TIMP-1 is secreted by keratinocytes. The latest study demonstrated that increased secretion of TIMP-1 by human HTS...
keratinocytes might contribute to fibrosis (Simon et al., 2012). Notch signaling, which is crucial in keratinocyte differentiation, after inhibition by DAPT (a Notch inhibitor), down-regulated the production of fibrotic factors in keratinocytes and ameliorated HTS formation (Li et al., 2016a). The changes of keratinocytes in HTS bring us another way to understand the mechanism of HTS, which needs further research.

**Cell activities are regulated by growth factors and signals**

Several growth factors including TGF-β, CTGF, platelet-derived growth factor (PDGF) and epidermal growth factor (EGF) all participate in HTS formation (Table 1). Of all the growth factors, TGF-β, which consists of TGF-β1, 2, 3, plays a pivotal role in HTS formation (Zhu et al., 2016a). Through the downstream signaling pathways involving SMAD pathway and non-SMAD pathway, TGF-β1 regulates proliferation, migration, trans-differentiation and collagen deposition of fibroblasts (Zhang, 2009; Penn et al., 2012; Lian and Li, 2016). HTS and resident fibroblasts show higher expression of TGF-b1 and lower levels of TGF-b3 than normal. (Xue and Jackson, 2015). TGF-β3, different from TGF-β1 and 2, suppresses fibroblast differentiation, ECM synthesis, and scar formation (Occleston et al., 2008; Chang et al., 2014). TGF-β has been investigated as a treatment target. Recent research using TGF-β receptor siRNA or antagonist peptide showed significant effects in treating HTS (Lian and Li, 2016). Besides, several phase I and phase II clinical trials of human recombinant TGF-β3 have shown significant improvement in scar (Berman et al., 2017).

CTGF (CCN2) is increased in almost all kinds of fibrosis diseases, including HTS (Lian and Li, 2016). CTGF inhibitor could limit HTS formation without affecting wound healing in a rabbit HTS model (Sisco et al., 2008). Studies from our group demonstrated that CTGF could induce collagen I and α-SMA expression in HTS fibroblasts, and is partly through integrin αβ3 and ERK/JNK signaling pathways (Hu et al., 2013, 2014). Recent study using nanolayered CTGF siRNA delivery platforms showed promising effect on reducing scar formation in a third-degree burns rabbit model (Castleberry et al., 2016; Mundy et al., 2016). Interestingly, CCN5, a member of the CCN family, showed an anti-fibrotic effect through suppressing CCN2-mediated fibrogenesis (Leask, 2010). In skin fibroblasts, transfection of adenoviral vectors carrying CCN2 or CCN5 respectively resulted in the opposite effect. Upregulation of CCN2 decreased CCN5 while promoted collagen and α-SMA expression. On the contrary, upregulation of CCN5 decreased CCN2, collagen and α-SMA expression (Xu et al., 2015). As a natural inhibitor of CTGF, CCN5 may be a potential treatment for HTS, which needs more investigation.

PDGF, secreted by macrophages, participates in wound healing by promoting fibroblast proliferation, migration and collagen deposition (Lian and Li, 2016). Besides, PDGF could also promote monocyte recruitment in the dermal fibrosis process (Cho et al., 2016). Recent studies focused on PDGF and its receptor indicate an attractive target for treatment of fibrosis diseases (Buhl et al., 2016; Lu et al., 2016; Wang et al., 2016a). EGF is an essential growth factor during the wound healing process, and participates in keratinocyte and fibroblast function and granulation tissue formation (Lian and Li, 2016). In HTS process, EGF plays a complicated role (Lian and Li, 2016). Recent clinical trials showed that the early use of recombinant human EGF in surgical wound healing significantly reduced the thickness and Vancouver scar scale score (Shin et al., 2015), suggesting a potential usage for HTS prevention. However, more evidence is needed for the effect of EGF in advanced HTS. Fibroblast growth factor (FGF) has been widely investigated for wound healing. Recently, it was found to benefit scar reduction. After treatment with FGF, fibroblasts derived from HTS showed decreased collagen and increased MMP1 production (Song et al., 2011; Eto et al., 2012). Human HTS tissue implanted into nude mice showed degradation of collagen fibers and higher MMP1 after FGF-2 treatment (Eto et al., 2012). Similar results were found in HTS rabbit ear model (Shi et al., 2013). More recently, Sideek et al. (2016) revealed increased expression and co-localization of FGF-2 with LTBP-2 in HTS tissues, suggesting that LTBP-2 might bind to FGF-2, which caused loss of anti-scarring function of FGF-2 (Sideek et al., 2016).

The formation of granulation is essential in deep wound healing process, and the blood vessels are one of the most important components for their function of bringing necessary cells and nutrients to the wound bed. In response to hypoxia, VEGF is produced and acts as the most effective pro-angiogenesis factor (DiPietro, 2016). In normal wound healing process, most

<table>
<thead>
<tr>
<th>Growth factors</th>
<th>Functions in HTS</th>
</tr>
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<tbody>
<tr>
<td>TGF-β</td>
<td>Inflammation, angiogenesis, fibroblast proliferation and trans-differentiation, collagen synthesis, deposition and remodelling of the ECM</td>
</tr>
<tr>
<td>CTGF</td>
<td>Cell proliferation and migration, myofibroblast differentiation and ECM production, downstream of TGF-β</td>
</tr>
<tr>
<td>FGF</td>
<td>Collagen degradation, inhibition of collagen production, inhibition of TGF-β1 pathway</td>
</tr>
<tr>
<td>PDGF</td>
<td>Chemoattractant for monocytes, proliferation, collagen synthesis and migration of fibroblasts, inflammatory reactions</td>
</tr>
<tr>
<td>VEGF</td>
<td>Angiogenesis, migration of fibroblasts and keratinocytes, regulation of macrophages</td>
</tr>
<tr>
<td>EGF</td>
<td>ECM degradation, wound contraction, epidermal proliferation, anti-inflammation, fibroblasts proliferation and migration</td>
</tr>
</tbody>
</table>
Mechanisms of hypertrophic scar

Inflammation plays a major role in the process of normal wound healing and HTS formation. It is believed that excessive inflammation is highly related to scar formation (Ud-Din et al., 2014; Qian et al., 2016). Wound repair in early fetal stage goes through non-inflammation process, and ends up with perfect repair with no scar (Redd et al., 2004). Using a rabbit ear model, Qian et al. (2016) found the injection in wounds with pathogen-associated molecular pattern and damage-associated molecular pattern stimulators, which could induce excessive and prolonged inflammation, resulted in increased scar formation than control group. Mechanical force could also lead to high risk of HTS formation accompanied by severe inflammatory reaction (Wong et al., 2012; Cremers et al., 2015). Another research with a rabbit ear model revealed that TSG-6 significantly suppressed HTS formation through its anti-inflammation function (Wang et al., 2015). Shi et al. (2014) found that IL-10, a major anti-inflammatory cytokine during wound healing process, has a pronounced anti-HTS effect. Besides the inhibition effect of IL-10 on collagen deposition (Shi et al., 2014), IL-10 was proved to inhibit HTS fibroblast autophagy through the IL10-IL10R-STAT3 and IL10-AKT-mTOR pathway, and provided a potential therapeutic agent for HTS treatment (Shi et al., 2016). However, two recent studies from one group demonstrated that HTS are related to a suppressed or delayed early immune and inflammatory response compared with NTS (van den Broek et al., 2015; Butzelaar et al., 2016b). In these studies, concentrations of IL-6, IL-8, and CCL2 of HTS were significantly decreased compared to NTS by measuring the biopsies taken 3 hours after human skin wound (surgery). In addition, HTS formation coincided with a decreased expression of inflammatory genes such as IL-1α, TNF-α, IL-1RN, CCL2, CCL3, CXCL2, CXCR3, C3 and IL-10. On the contrary, ECM-related gene expression was increased in HTS, as well as the prolonged infiltration of M2 macrophages. Besides, recently prostaglandin was found to play an anti-scar effect, as a classical pro-inflammation mediator (Zhao et al., 2016). These results make the relationship between inflammation and HTS more controversial. Thus, it is important to further investigate the inflammatory factors in more detail rather than regarding them as a whole.

Mast cells, as resident inflammatory cells, also participate in the whole wound healing process. Mast cells could affect fibroblasts, keratinocytes, and wound angiogenesis through all kinds of growth factors including EGF, keratinocyte growth factors (KGF), VEGF, PDGF and FGF-2. Besides, mast cells could also directly interact with fibroblasts by gap junction, through which mast cells could promote the proliferation, differentiation and collagen deposition of fibroblasts (Wilgus and Wulff, 2014). Studies in fetal wound model found that scar formation was highly related to growing numbers of mast cells (Wulff et al., 2012). Blockade of mast cells with disodium cromoglycate, a mast cell inhibitor, reduced scar formation while accelerating collagen re-organization without influence on re-epithelialization or tissue strength, providing a potential

Microenvironment: inflammation and immunity

Inflammation plays a major role in the process of normal wound healing and HTS formation.
treatment for HTS (Chen et al., 2014b). However, there are also several studies claiming that mast cells have limited effect on skin fibrosis (Nauta et al., 2013; Willenborg et al., 2014). Besides, mast cells were shown to cause pain and pruritus in HTS (Kwak et al., 2016). More research is needed to investigate the relationship between mast cells, neurogenic inflammation and fibrosis in HTS.

Macrophages are thought to play a critical role in wound repair and fibrosis (Wynn and Vannella, 2016). It is believed that M1 and M2, which represent the two extremes of a series of macrophages with different functions, have distinct opposite functions in the wound healing process (Delavary et al., 2011; Sica et al., 2015). M1 macrophages produce pro-inflammation cytokines (TNF-α, IL-1β, IL-6, and IL-23), while M2 macrophages are key effectors of Th2 responses, anti-inflammation and tissue repair, and will lead to fibrosis when prolonged. Evaluation of scar tissue collected from patients revealed a high number of macrophages in HTS compared with NTS (Amini-Nik et al., 2014). By systemic depletion of macrophages in a mouse HTS model, Zhu et al. (2016b) found the scar formation was reduced compared with control group (Zhu et al., 2016b). MCP-1 knockout mice exhibited reduced scar formation, in company with less recruitment of macrophages (Wong et al., 2012). Another chemokine of monocytes showed a similar effect in human HTS-like nude mouse model (Ding et al., 2014). van den Broek et al. (2015) also found an increased number of macrophages, which are mainly M2 macrophages in HTS compared with NTS (van den Broek et al., 2015; Butzelaar et al., 2016b). Besides, β-catenin-mediated macrophage motility contributes to the number of mesenchymal cells and ultimate scar size following cutaneous injury. These studies may partly explain the suppressed inflammation mentioned above in HTS, considering the increased number of M2 macrophages and their anti-inflammation character. The further work should focus on the reason for the change of macrophages in HTS, which could be a potential therapeutic target.

Effective healing is usually characterized by a dominant Th1 response, whereas a predominant Th2 response and an increase of Th17 cells lead to chronic inflammation, which can ultimately result in fibrosis (Georg et al., 2013). A recent article showed that at 3 days after burn, the T cells in wound site demonstrated Th2 and Th17 predominant phenotypes (Rani et al., 2014). Thus, it is possible that HTS formation after burn is caused by the prevalence of Th2 and Th17. An experiment using a murine model of HTS induced by

Mechanisms of hypertrophic scar

Fig. 2. Mechanotransduction in HTS fibroblasts/myofibroblasts. Several signal pathways participate in the transduction of mechanical force to cell activities. The most important effectors are integrins, which induce autophosphorylation under the stimulation of mechanical forces, followed by downstreams such as PI3K and MAPK. Besides, integrin affects LAP and releases TGF-β, enhancing TGF-β-SMADS pathways. Calcium channels and G proteins coupled receptors and small G proteins such as Rho also take part in mechanotransduction.
mechanical force demonstrated that T cells were critical for the inhibition of inflammatory cells and HTS formation during mechanical response, and the mechanical stimulation is Th2 cytokine and chemokine related (Wong et al., 2011). This confirmed the significant effect of T cells in HTS formation. IL-4, as a typical type-2 cytokine, was proved to organize the profibrotic dihydroxy lysinonorleucine-collagen crosslink, which was critical for scar formation through the activation of M2 macrophages by IL-4 receptor α (Knipper et al., 2015). Th17 cells, a recently known T cell subset, were reported to affect the process of fibrosis by its pro-inflammation property and directly stimulation to fibroblasts/hepatic stellate cells (Meng et al., 2012; Fabre et al., 2014; Lei et al., 2016). In many cases, IL-17A expression is associated with persistent neutrophils, and it has been suggested that exaggerated neutrophil recruitment contributed to the tissue damage and fibrosis by inducing apoptosis of vascular endothelial cells (Zhu et al., 2011). However, the exact role of Th17 and IL-17 on fibrosis and HTS need to be further researched. T regular cells (Tregs) are important for immunosuppression, and recent studies suggested their complicated relationship with fibrosis diseases. Keloid tissues were shown to contain less Tregs compared with other inflammatory skin conditions, and Tregs reduced the collagen synthesis by keloid fibroblasts in a coculture system (Murao et al., 2014). In cardiac, pulmonary fibrosis and systemic sclerosis (Cao et al., 2013; MacDonald et al., 2015; Xiong et al., 2015a), Tregs showed different effects on fibrosis. Besides, the balance of Th17 and Tregs also participates in the fibrosis process, which is still controversial (Georg et al., 2013; Galati et al., 2014; Xiong et al., 2015b). The exact effects of Tregs and Th17 in HTS need to be investigated (Fig. 2).

Promising treatment: Mesenchymal stem cells and fat tissue transplantation

Mesenchymal stem cells (MSCs) have been researched for decades (Brunet et al., 2012). There is increasing evidence showing that MSCs could inhibit fibrosis and decrease scar formation (Li et al., 2016c). Induced pluripotent stem cells (iPSCs), as embryonic-like stem cells, were shown to inhibit hypertrophic scar (Ren et al., 2015). With iPSC-conditioned medium (iPSC-CM), the expression of collagen I and α-SMA in HTS fibroblast was reduced significantly. iPSC-CM could also reduce the contractility of fibroblasts and block the recruitment and adhesion of inflammatory cells. In a rabbit ear HTS model, bone marrow derived MSCs (BMSCs) injection through ear artery inhibited HTS formation, which was abrogated by p53 gene knockdown. This study demonstrated that BMSCs affected HTS formation at least in two ways, including inhibition of proliferation and trans-differentiation of fibroblasts, both of which were p53 dependent (Liu et al., 2014b). Another research found similar results and further proved the change of fibrosis-related genes induced by BMSC-conditioned medium (Fang et al., 2016). On a bleomycin-induced skin fibrosis mice model, BMSC alleviated inflammation, promoted the remodeling of ECM and eventually ameliorated fibrosis induced by bleomycin (Wu et al., 2014). This article also suggested that BMSC injected subcutaneously disappeared within 24 hours, providing useful tips for further investigation. Interestingly, another group found that the fast disappearance of BMSCs was necessary for its anti-inflammation activity (Liu et al., 2014c). They demonstrated that after injection, BMSCs underwent extensive apoptosis, which promoted TSG-6 secretion, and followed by inhibited inflammation and reduced scar formation. And this process was caspase-3 dependent. However, embryonic stem cells (ESCs) derived macrophages were proved to delay wound healing and promote scar formation. One of the reasons might be that macrophages derived from ESCs exhibited M2-like phenotype, which promoted fibrogenesis (Dreymueller et al., 2013).

Fat grafting has been used worldwide for treatment of burns and other difficult wounds. It has influenced the way of treatment of burns, burn scars, and other difficult wounds (Piccolo et al., 2015). Autologous fat grafting provides beneficial effects with limited side effects. Also, it shows statistically significant improvement of the scar appearance, reduction of pain, itch and restoration of volume and three-dimensional contour (Negenborn et al., 2016). A clinical trial including 93 patients with burn scars observed a remarkable improvement in function and aesthetic after treatment with autologous fat grafting compared with control group (Bruno et al., 2013). It is also interesting that, both in human and animal models, fat graft has the effect of alleviating neuropathic pain in burn scar (Huang et al., 2015; Fredman et al., 2016), and is partly due to its anti-inflammation effect. In order to clarify the mechanism, recent studies transferred the concern from fat tissue to adipose derived stem cells (ADSCs). ADSCs are autologous, non-immunogenic, easily available in large quantities, and seem to be a promising approach for wound repair and anti-scar therapy. A study in a pig scar model found that local injection of ADSCs decreased scar size and provided better color quality and scar pliability. Besides, it decreased the activity of mast cells and inhibited the action of TGF-β against fibroblasts and positively stimulated scar remodeling through greater expression of MMPs (Yun et al., 2012). Studies also found that ADSCs had the effect of improving wound healing speed and angiogenesis while reducing contraction in animal models (Uysal et al., 2014; Rodriguez et al., 2015). A study in a rabbit ear HTS model revealed that an intralesional injection of ADSCs reduced the formation of rabbit ear HTS by decreasing the expression of α-SMA and collagen type I genes and ameliorating collagen deposition and this may result in an effective anti-scarring therapy (Zhang et al., 2015b). The research from our group partly explained the
mechanism of ADSCs’ effect. We demonstrated that the p38/MAPK pathway participated in the process of anti-fibrosis effect of ADSC in vivo and in vitro (Li et al., 2016d). Recent study indicated that ADSC could affect scar formation by exosomes, which needs further research (Hu et al., 2016).

Conclusion

Hypertrophic scars are caused by burns, surgery and other skin injuries. The main difference between HTS and normal scar is the excessive ECM deposition. In this process, fibroblasts/myofibroblasts are the most important cells. The growth factors, mechanical forces, inflammatory and immune responses lead to the abnormal activity of fibroblasts/myofibroblasts. Keratinocytes and angiogenesis also participate in HTS formation. Mesenchymal stem cells and fat tissue transplantation are emerging treatments for HTS. A better understanding of the mechanism is needed to lead us to more effective prevention and therapeutic strategy.

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