Adipose-Derived Tissue in the Treatment of Dermal Fibrosis

Antifibrotic Effects of Adipose-Derived Stem Cells

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Abstract: Treatment of hypertrophic scars and other fibrotic skin conditions with autologous fat injections shows promising clinical results; however, the underlying mechanisms of its antifibrotic action have not been comprehensively studied. Adipose-derived stem cells, or stromal cell–derived factors, inherent components of the transplanted fat tissue, seem to be responsible for its therapeutic effects on difficult scars. The mechanisms by which this therapeutic effect takes place are diverse and are mostly mediated by paracrine signaling, which switches on various antifibrotic molecular pathways, modulates the activity of the central profibrotic transforming growth factor β/Smad pathway, and normalizes functioning of fibroblasts and keratinocytes in the recipient site. Direct cell-to-cell communications and differentiation of cell types may also play a positive role in scar treatment, even though they have not been extensively studied in this context. A more thorough understanding of the fat tissue antifibrotic mechanisms of action will turn this treatment from an anecdotal remedy to a more controlled, timely administered technology.

Key Words: adipose-derived stem cells, fat injection, fibrosis, hypertrophic scar, scar treatment, TGF-β, Smad, wound healing

(*Ann Plast Surg* 2018;00: 00–00)

Since the wide clinical introduction of fat grafting in the 1990s,[1,2] applications of this primarily aesthetic surgical tool have become numerous and today extend into the reconstructive field. One of the first exciting reports that fat can be used not only as a masking agent, but also as a truly curative tool, was presented by Rigotti et al,[3] who investigated the applicability of fat grafting for the treatment of radiation therapy complications characterized by pronounced tissue fibrosis and atrophy. In a group of 20 patients, treatment with autologous fat injection resulted in clinical improvement even in cases classified as having irreversible tissue damage and loss of function.[3]

Since then, evidence of the positive clinical effects of fat grafting for scar/fibrosis treatment has been confirmed by multiple clinical observations.[4–22] Klinger's group has used fat injections for the treatment of different types of scars characterized by a pronounced fibrotic tissue component.[11] The authors paid specific attention to patients with hypertrophic and keloid scars resulting from burns. An injection of autologous fat tissue to the dermohypodermal junction of the scar area in 2 sessions, separated by 3 months, resulted in an improved scar texture, softness, thickness, and elasticity.[5] Similar results were observed by other authors using fat grafts for the treatment of postburn complications.[10,13,22]

Fat grafting for fibrotic treatment has also been used for scars resulting from trauma,[12,15,19] surgery,[4,15–17,19] acne, and abscess formation.[19] Specifically, fat transplants are effective in the functional and aesthetic restoration of the face and body when they are used for the treatment of scars causing functional problems such as ectropion,[4] microstomia, lip eversion, nasal valve collapse, and joint contractures.[15] In addition, it corrects painful and retracted scars from tracheostomy,[16] can be used to treat pain and restore sexual function in patients suffering from perineal and vaginal scars as a result of childbirth/episiotomy,[12] and can effectively prepare thoracic tissues for breast reconstruction after mastectomy by improving their quality and pliability and making them more suitable for implant placement, tissue expanders, and/or autologous tissue flaps.[8,15] Fat grafting has also been used to treat postmastectomy pain syndrome caused by scar tissue nerve compression.[18] Autologous fat is also used for total reconstruction of the breast after mastectomy and radiation therapy, where the tissue is characterized by severe fibrotic changes.[14] The use of fat tissue to treat difficult scars is enticing because the fat is readily available and easily accessible, and the procedure is minimally invasive and can be done under local anesthesia with a short downtime compared with some traditional surgical methods for scar correction.

Another relatively new area of fat grafting use is in the treatment of fibrotic diseases/processes that are traditionally considered to be an area of interest for plastic surgeons. Fat injection, in conjunction with percutaneous aponeurotomy, is effective for the treatment of Dupuytren contracture, with restoration of a supple subcutaneous fat pad, a more healthy appearance of the palmar skin, and, most importantly, a good functional result in most patients.[9] Good clinical outcomes are also found for fat grafting in conjunction with secondary carpal tunnel release[21] and tenolysis,[7] although relatively little evidence has been gathered in these areas. Furthermore, treatment with autologous fat is being explored as a method of alleviating dermal fibrotic changes in systemic sclerosis.[20]

Despite encouraging results, the diversity of uses, and the yet expanding clinical knowledge of fat grafting applications for scar/fibrosis treatment in plastic surgery, there still remains a huge gap in understanding the underlying mechanisms of the improved tissue quality after fat grafting. Most researchers appreciate the fact that the positive changes brought about by fat injections are not due to the volumetric effect of the injection itself, as the volume of the transplanted tissue is usually quite small compared with the overall area of the treated defect.[4] Moreover, the simple addition of a passive filler would not explain the positive changes in the scar tissue texture, color, and elasticity, as described in the previously mentioned studies. Therefore, the encouraging results have been linked rather to the presence of adipose-derived stem cells (ADSCs) in the fat graft and to the ability of these cells to proliferate and differentiate into various cell lineages,[5,13] as well as the secretion of soluble factors by the ADSCs and their interactions with the inherent tissue components of the scar.[3,17,22] However, all of these considerations are largely speculative to date, and fat grafting research has not yet provided a widely accepted explanation of the antifibrotic action of ADSCs.

Thus, the aim of this review is to identify the molecular/cellular mechanisms underlying the antifibrotic effects of transplanted fat and/or ADSCs as its component. This evidence will provide a better background for understanding the effect of fat grafting in the setting of difficult scars and fibrotic diseases.

Characterization of Scar/Fibrotic Tissue

The closure of wounds in postnatal life is achieved by wound contraction and the formation of scar tissue, which is characterized by a

Received August 8, 2017, and accepted for publication, after revision September 22, 2017.
From the Center for Tissue Engineering, University of California, Irvine, Irvine, CA.
Conflicts of interest and sources of funding: none declared.
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ISSN: 0148-7043/18/0000–0000
DOI: 10.1097/SAP.0000000000001278

robust deposition of newly synthesized extracellular matrix (ECM).[23] The wound-healing process is classically divided into 4 overlapping phases of hemostasis, inflammation, proliferation, and remodeling.[23–25] One of the central players in wound healing is the fibroblast. This type of cell promotes healing and scar formation by secreting various paracrine factors and differentiating into myofibroblasts. These are highly specialized contractile cells that are characterized by the expression of α smooth muscle actin (α-SMA) and function to synthesize collagen and other components of the provisional ECM, as well as bring the wound edges together by contractile forces. After the scar maturation is complete, the myofibroblasts normally undergo apoptosis. Some of the important factors orchestrating wound healing via the secretion of ECM components include interleukin 1β (IL-1β), IL-18, transforming growth factor β (TGF-β), epidermal growth factor, and fibroblast growth factor (FGF).[24]

This normal process of wound healing is transient, with most wounds taking no longer than 2 to 3 weeks to heal.[26] However, certain types of wounds, such as deep and deep partial-thickness burns, infected wounds, and wounds healing by secondary intention, are prone to pathologic healing with the formation of hypertrophic scars (HTSs) that are characterized by extensive fibrosis. It is thought that this fibrotic process is due to the dysregulation of wound healing in either the proliferative or the remodeling phase.[24,27] Normally, cytokine signaling in wound healing is tightly regulated, whereas the dysregulation causes an uncontrollable secretion of cytokines, leading to the continuous production of ECM components.[24] The sustained release of various components of the wound-healing cascade has been linked to hypertrophic scarring. Specifically, the dysregulation of caspase 1, IL-1β, IL-18, IL-8, TGF-β1, stromal cell–derived factor 1, monocyte chemoattractant protein 1, FAK (focal adhesion kinase), and Toll-like receptor 4 has all been implicated in the formation of pathologic scars.[24,25]

Morphologically, HTSs present as raised red scars that are confined to the borders of the original wound. Hypertrophic scars are characterized by pain and can be aesthetically disfiguring, especially when they arise in exposed parts of the body. The histological features of HTSs include a thickened epidermis with flattening of the rete ridges,[23] an increased proliferation of keratinocytes,[25] and an increased thickness of the dermis with hypercellularity characterized by a large number of fibroblasts and myofibroblasts, hypervascularity, and abnormal collagen organization.[23,28] Collagen is highly abundant in pathologic scars[28] and is disorganized with thick fibers of irregular shapes[25] that can form nodal structures.[23] An important feature of scar tissue is the absence of the basement membrane (BM) with a reduction in expression of collagen type IV.[25] The BM is more than just a passive ECM structure: it serves as a communication interface between dermal fibroblasts and epidermal keratinocytes and favors epidermal-dermal homeostasis[25,29,30]; therefore, its disruption in certain types of wounds is thought to promote fibrosis.[25]

As discussed previously, other types of fibroproliferative conditions such as radiation-induced tissue fibrosis, Dupuytren disease, and fibrotic changes to the skin in systemic scleroderma are also of interest to plastic surgeons and show similar promising results with fat grafting treatment. Even though the triggering events of these conditions are different from the previously described process of pathological wound healing with HTS formation, the pathogenesis and histological features of the resulting fibrosis have much in common. For instance, the development of Dupuytren disease is mediated by aggressive myofibroblasts expressing highly contractile α-SMA, which are responsible for both an excessive ECM deposition and tissue contraction.[31] Radiation-induced skin fibrosis is characterized macroscopically by skin discoloration and loss of elasticity and microscopically by microvascular obliteration, which is a consequence of external radiation, and dermal thickening.[3,32]

Changes in Fibrotic Tissue After Fat Grafting

Most authors refer to the histological changes observed in scars after fat grafting as "regeneration" or a return to normal tissue architecture.[10,13] The treated area is characterized as a "reappearance" of the papillary dermis[13] with a reduction in dermoepidermal junction flattening, a restoration of a normal ridge pattern, and epidermal crest elongation.[20] That being said, the dermal/skin thickness is actually decreased with treatment.[32–34] Fat grafting leads to a decrease in the dermal collagen content[32–36] with a looser and better organized pattern of the residual collagen fibers.[13,37] However, Brongo et al[10] report new collagen deposition in the context of tissue regeneration. In addition, the expression of α-SMA seems to be decreased.[37] Other histological features after fat graft treatment of scarred tissue include an increased number of elastic fibers in the dermis and a diminished number of melanocytes and Langerhans cells.[13] An injection of fat somewhat normalizes the abnormal vascularization pattern of scar tissue by selectively increasing vascularity[10,13,20,32,34] and normalizing microvascular architecture.[13,20]

Possible Mechanisms of ADSC Antifibrotic Effect

To date, only a limited number of studies have focused on the antifibrotic mechanisms of ADSCs in the setting of cutaneous/subcutaneous scar treatment. This is partially due to the absence of an established in vitro model for this pathologic process. In the present section, we summarize the molecular mechanisms of scar attenuation by ADSCs that have been investigated, as well as propose other possible mechanisms based on ADSC research in other clinical fields (Fig. 1).

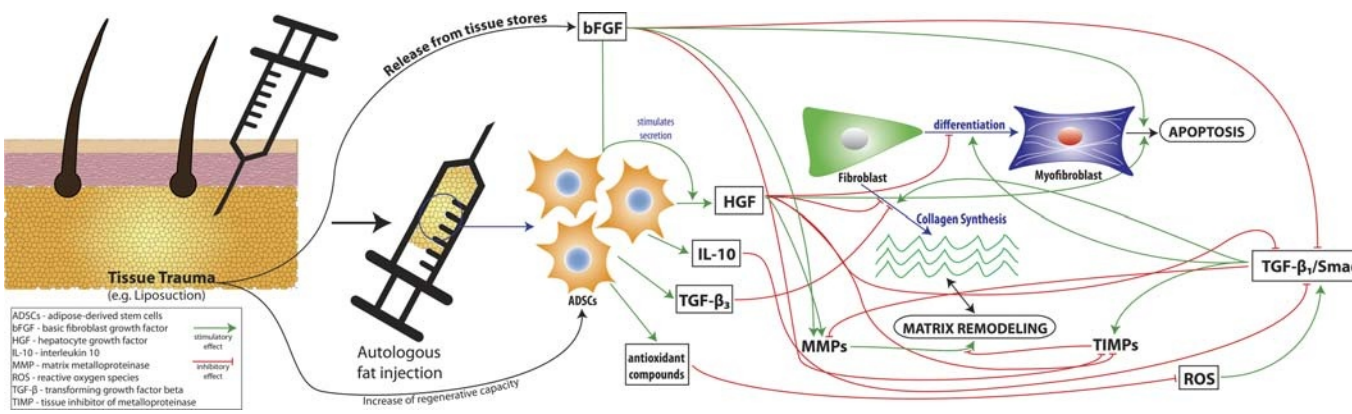

FIGURE 1. Paracrine mechanisms of ADSC antifibrotic action: interactions with the TGF-beta1/Smad axis and its downstream matrix remodeling and wound contracting consequences.

Interactions With the TGF-β/Smad Axis

The TGF-β/Smad axis is one of the central players in the wound-healing cascade, and dysregulation of this pathway leads to pathological patterns of healing, such as those seen in excessive scarring conditions.[38,39] A decreased expression of TGF-β1 is recognized as an important consequence of ADSC administration for fibrosis treatment in multiple studies.[36,40–44] However, the interactions of ADSCs with members of the TGF-β family seem to be far more complex than a simple 1-way inhibition.

Transforming growth factor β cytokines elicit their effects through 2 types of transmembrane receptors, known as types I and II receptors (TβRI and TβRII). Stimulation of these receptors activates intracellular Smad transcription factors (Smad2 and Smad3), which mediate phosphorylation with Smad4 allowing entry into the nucleus and regulation of gene transcription.[38,45] While the TGF-β superfamily comprises more than 30 ligands, which have been shown to play diverse roles in embryogenesis, tissue homeostasis, disease, and cancer development,[38,46–49] we are primarily interested in the TGF-β subfamily, which consists of 3 TGF-β isoforms: TGF-β1, TGF-β2, and TGF-β3. The 3 isoforms have specific and different effects during wound healing. Adult tissues predominantly express TGF-β1, whereas TGF-β3 is the main isoform present prenatally.

During the wound-healing process, the latent form of TGF-β is activated biphasically. The first portion of TGF-β1 is released from activated platelets within minutes after injury,[50,51] whereas TGF-β2 and TGF-β3 slowly rise over the first 24 hours after the insult.[52] In the inflammatory stage of wound healing, TGF-β1 works to recruit neutrophils and monocytes to the site of injury and directs monocytes into activated macrophage differentiation.[38,53] Interestingly, as inflammation subsides, activated immune cells become susceptible to TGF-β1–dependent inhibition, thereby demonstrating the duality of the function of TGF-β1 and its temporal dependence.[53] The second phase of TGF-β1 activation occurs in the proliferative stage during re-epithelialization,[52] when it orchestrates angiogenesis, promotes fibroblast-to-myofibroblast differentiation, increases the synthesis of provisional matrix components, and stimulates keratinocyte migration. In addition to directly stimulating collagen and fibronectin synthesis, TGF-β1 also inhibits the ECM breakdown enzymes known as matrix metalloproteinases (MMPs), and the increased expression of the opposing tissue inhibitors of metalloproteinase (TIMPs) is a well-recognized mechanism by which TGF-β1 favors ECM formation.[38] The transition into the remodeling stage of wound healing depends on establishing a pattern of the ongoing synthesis and degradation of collagen at a low rate and thus is largely affected by TGF-β1 as a major regulator of MMP and TIMP expression, as well as a promoter of collagen cross-linking.[38,53] Elevated levels of TGF-β1 are a characteristic feature of HTSs.[38] It seems that the continuous activation of TGF-β1/Smad signaling mediates the excessive production of ECM with a lack of appropriate remodeling.[39]

The extensive role of TGF-β1 in HTS pathogenesis has logically driven attention to developing a treatment that would affect the TGF-β1/Smad axis in order to achieve more favorable scarring outcomes. While many pharmacological and genetic therapeutic options are being tested, none of these have yet proven to be effective enough and approved for use as a criterion standard of HTS treatment.[38] As discussed previously, the first clinical results of autologous fat/ADSC use for fibrosis treatment are extremely promising, and lower levels of TGF-β1 in the scar tissue were confirmed after such treatments. In a study by Spiekman et al,[54] TGF-β1–treated human dermal fibroblasts exhibited increased proliferation, gene up-regulation, protein expression of the contractile marker SM22α, collagen gel contraction, extracellular deposition of collagens I and III, and a hypertrophic morphology, all of which were reduced by treatment with ADSC-conditioned medium (ADSC-CM). Furthermore, ADSC-CM promoted the expression of MMPs, namely, MMP-1, MMP-2, and MMP-14. Altogether, ADSC-CM seemed to normalize not only HTS cell morphology, but also the turnover of the ECM, making it resemble the normal remodeling process. The authors thus conclude that normalization of the TGF-β1–induced hypertrophic features of dermal fibroblasts is the primary mechanism underlying the positive scar resolution effects seen with fat grafting.[54]

Another member of the TGF-β subfamily, TGF-β3, is the predominant isoform present in prenatal life, and it has been linked to scarless healing in the fetus, as well as a decreased scar formation without the reduction in scar tensile strength in adult wound healing.[38,51,55] It is suggested that changing the TGF-β1/TGF-β3 ratio toward increasing TGF-β3 in the wound milieu favors reduced scarring, and recombinant TGF-β3 has been developed and used clinically for this purpose.[55] An increased expression of TGF-β3 with stem cell treatment was demonstrated using in vitro and in vivo models. Yun et al[40] showed a transient increase in TGF-β3 levels after ADSC injection in an HTS pig model. However, TGF-β3 has not been the focus of research in the area of ADSC-mediated fibrosis treatment to date, even though it has been studied in the context of the antifibrotic effects of bone marrow–derived stem cells (BMSCs). Huang et al[56] cocultured murine dermal fibroblasts with BMSCs in an inflammatory environment and found that this led to an increased expression of TGF-β3 among other cytokines, which correlated with a reduced proliferation of fibroblasts and decreased TGF-β1 and collagen synthesis compared with an inflammatory stimulation of fibroblasts alone. Another study by Wu et al[57] also demonstrated that BMSC-conditioned medium contains high levels of TGF-β3 and that blocking TGF-β3 activity in BMSC-conditioned medium cancels its antifibrotic effects in human keloid fibroblasts.

When discussing the effects of ADSCs on TGF-β expression, one has to take into consideration the fact that the communication is bidirectional; for example, TGF-β cytokines can in turn impose their own effects on ADSCs. The research results are somewhat confusing as the direct stimulation of ADSCs with TGF-β1 seems to render them with potentially fibrotic inducing features. According to Desai et al,[58] ADSCs treated with TGF-β1 acquire a "myofibroblast" phenotype, presenting as spread cells with robust focal adhesions and pronounced actin stress fibers. These cells show an increased expression of α-SMA and Smad2 and an increased synthesis of collagen type I and fibronectin. They also demonstrate the capacity to contract the matrix in vitro. Thus, it seems that exposure of ADSCs to TGF-β1 leads to their differentiation into myofibroblast-like cells, which is putatively accomplished through the Smad2 signaling mechanism. The simple removal of TGF-β1 from the surrounding environment surprisingly leads to an increase in α-SMA synthesis, which contributes to the intrinsic ability of myofibroblast-like cells to produce α-SMA. This phenotype is, however, reversible because treatment of "myofibroblasts" with basic fibroblast growth factor (bFGF) leads to a decrease in cell hypertrophy, a decrease in α-SMA, and the loss of Smad2 expression. The previously noted ability to contract the matrix is reduced while the cells seem to be more mobile (see Paracrine Mechanisms for more details).[58] Nevertheless, the potential myofibroblast differentiation of ADSCs influenced by TGF-β1 may present a concern in the context of ADSC-based scar/fibrosis treatment, as the ongoing fibrotic process is characterized by high baseline levels of TGF-β1 in the injection area.

Myofibroblast differentiation of ADSCs after treatment with TGF-β1 in vitro was also shown in a study by Kakudo et al.[59] In this study, cells demonstrated the expression of intracellular actin stress fibers and α-SMA, the induction of collagen gel contraction, and an increased expression of collagen types I and II and fibronectin. The motility of these "myofibroblasts," however, was decreased compared with baseline, which is contrary to the findings by Desai et al.[58] In another study by Cho et al,[60] human skin fibroblasts were treated with conditioned medium from TGF-β1–stimulated ADSCs, which resulted in increased fibroblast proliferation and migration and increased collagen I and MMP-1 expression. Both studies concluded that TGF-β1 stimulation is a potential tool for promoting wound healing with ADSC

treatments, although neither of these studies investigated the potential outcomes of such treatment in terms of scar formation. As findings from these studies present features that are potentially beneficial for normal wound healing with balanced scar formation (eg, the increased motility of fibroblasts and differentiated ADSCs and the induction of MMP-1 along with collagen synthesis), we speculate that ADSC injection can thus be beneficial in the setting of a healing wound that is at high risk of developing HTSs by restoring the normal wound-healing conditions and promoting faster healing that would lack a prolonged remodeling phase (see Normalization of the Wound-Healing Process for more details). One way or another, the results of these studies are applicable to in vitro ADSC stimulation by TGF-β1, whereas these results are not necessarily translatable in vivo, for example, in a situation when ADSCs would be injected into fibrous tissue with high preexisting levels of TGF-β1.

Paracrine Mechanisms

It is well known that ADSCs are capable of secreting a vast range of soluble factors mediating their paracrine effects in enhancing wound healing.[39,61–63] The release of specific growth factors that promote a timely and adequate tissue repair by ADSCs is likely due to the fact that this release is itself triggered by adipose tissue trauma and is thus aimed at the restoration of tissue integrity and homeostasis.[64] Autologous fat grafts used for injections in plastic surgery are obtained by liposuction, which presents a severe traumatic insult to the fat tissue and thus initiates the wound-healing cascade in which inherent ADSCs seem to play a key role. Our group studied the effects that mechanical stress poses upon the cells of the lipoaspirate and found that it significantly up-regulates multipotent and pluripotent markers of these cells, possibly increasing their regenerative capacity.[65] It also has been shown that mechanical injury to human adipose tissue promotes the release of FGF-2 (also known as bFGF), TGF-β, epidermal growth factor, and platelet-derived growth factor (PDGF) in the early postinjury period with a subsequent increase in vascular endothelial growth factor (VEGF) and hepatocyte growth factor (HGF) secretion toward the end of the first week.[66] However, as noted previously, it seems that not only do ADSCs secrete growth factors, but they also serve as a target for their downstream effects. Suga et al[64] precisely studied the effects of injury-associated growth factors on ADSC behavior. They demonstrated that treatment with bFGF promoted ADSC proliferation in a dose-dependent manner and led to a dramatic increase in HGF secretion, with both

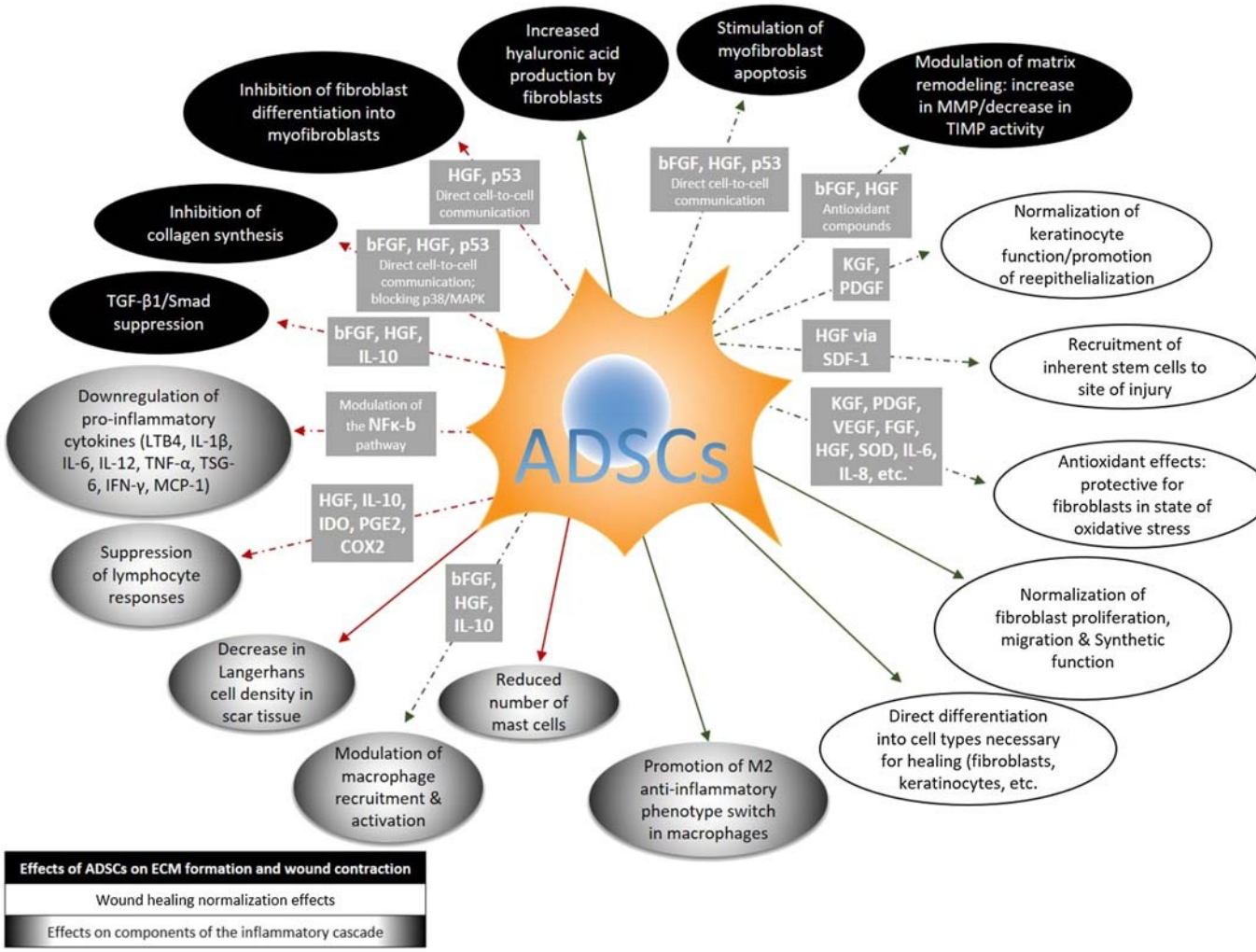

FIGURE 2. The main cellular and molecular effects of ADSC introduction into a fibrotic environment and their putative mechanisms. Black background balloon - effects on ECM formation and wound contraction; white background balloon- wound healing normalization effects; black shadow balloon - effects on components of the inflammatory cascade.

effects being mediated through the c-Jun N-terminal kinase (JNK) signaling pathway. An in vivo study further confirmed the presence of bFGF in adipose interstitial tissue early after injury, with a subsequent decline in bFGF levels and the concomitant rise in HGF.[64] This pattern suggests that trauma likely triggers bFGF release from tissue stores (such as ECM or dying cells), rather than its production de novo by activated ADSCs. A delayed increase in HGF concentration, preceded by a peak in bFGF levels, supports the previous in vitro findings (HGF synthesis by ADSCs is stimulated by bFGF). The role of the JNK signaling in this pathway was confirmed by an inhibition assay.[64] c-Jun N-terminal kinase is a messenger that belongs to the mitogen-activated protein kinase (MAPK) family of signaling molecules that play an important role in wound healing.[67–69] Furthermore, blocking JNK, bFGF, or HGF signaling leads to increased fibrogenesis in an ischemia-reperfusion tissue injury model, indicating that the bFGF-JNK-HGF pathway is antifibrotic[64] (Fig. 2).

The antifibrotic effects of both bFGF and HGF were reported in various organs. Basic FGF is a well-known agent that favors wound healing and reduces scarring.[70–73] The mechanisms involved may include the induction of myofibroblast apoptosis[73,74] through the Rho/Rho kinase pathway,[72] the inhibition of the TGF-β1/Smad–dependent pathway[73] with a resulting reduction in α-SMA expression,[75] the regulation of collagen balance and degradation[73,76] and overall ECM metabolism presumably by increasing MMP-1[77] and decreasing TIMP-1 expression,[73] and the modulation of macrophage activity.[73] Pretreatment of ADSCs with bFGF or a coinjection of ADSCs and bFGF is also a viable therapeutic option to reduce liver fibrosis,[78,79] promote endothelial regeneration and angiogenesis in limb ischemia,[80,81] and improve myocardial remodeling and cardiac function after myocardial infarction.[82] The stimulation of HGF release by ADSCs is also a well-known downstream effect of bFGF.[73,79] In a previously mentioned study by Desai et al,[58] bFGF was capable of reversing the TGF-β1–induced myofibroblast phenotype of ADSCs in vitro, presumably via the activation of the ERK (extracellular signal–regulated kinase)/MAP kinase pathway. Importantly, the authors demonstrated that simply blocking the TGF-β1 pathway does not reverse the myofibroblast phenotype on its own, and the addition of bFGF is necessary for the cells to redifferentiate.[58]

As for HGF, its antifibrotic effects are involved in the attenuation of myocardial,[83,84] pulmonary,[85–89] hepatic,[90,91] and renal[92–97] fibrosis and vocal cord scarring.[98] Similar to bFGF, HGF attenuates fibrosis by blocking TGF-β1–dependent matrix overproduction[96,99] and fibroblast-to-myofibroblast differentiation.[95,100] It also induces myofibroblast apoptosis through the FAK-ERK-MMP signaling cascade and promotes matrix remodeling by activating MMP-1, MMP-2, and MMP-9.[96,101] Hepatocyte growth factor interferes with the TGF-β1/Smad–dependent pathway by blocking the nuclear translocation[93,95] and promoting the nuclear export of Smad.[99] In a model of developing kidney fibrosis, HGF gene treatment or exogenous HGF administration leads to a significant decrease in a vast range of proinflammatory cytokines, including tumor necrosis factor α (TNF-α), interferon γ, monocyte chemoattractant protein 1, and IL-12, and was shown to prevent monocyte/macrophage and B-/T-cell infiltration.[92,94,95] These anti-inflammatory effects of HGF are accomplished through a reduction in nuclear factor κB activation,[94,102] a pathway that plays a major role in regulating inflammatory responses in mammalian cells.[103] Hepatocyte growth factor also promotes the recruitment of additional stem cells to the site of damage via the expression of stromal cell–derived factor 1.[104] Quite in line with the previously mentioned study published by Suga et al,[64] HGF expression is triggered by tissue injury and the resulting proinflammatory environment.[56,95] However, a chronic inflammatory milieu eventually decreases endogenous HGF production with a concomitant increase in TGF-β1 levels, favoring fibrosis.[95] Thus, exogenous HGF would be necessary in the setting of chronic inflammation/fibrosis in order to disrupt the vicious TGF-β1 cycle. Suga and colleagues' findings are further supported by a study by Kumai et al,[105] who demonstrated via a coculture experiment that the HGF released by ADSCs inhibits collagen synthesis in scar fibroblasts.

Another paracrine factor secreted by ADSCs and potentially involved in their scar-resolving effects is the anti-inflammatory cytokine IL-10.[37] It plays a pivotal role in wound-healing regulation by secreting anti-inflammatory compounds and controlling the recruitment and activation of inflammatory cells.[28] Experiments show that IL-10 attenuates lung fibrosis in vivo by inhibiting the production and activation of TGF-β1.[106] Shi et al[107] demonstrated that IL-10 significantly down-regulates the expression of collagen types I and III and α-SMA and up-regulates the expression of MMP-1 and MMP-8 in human fibroblasts pretreated with TGF-β1. Moreover, the number of α-SMA–positive fibroblasts decreased, and collagen lattice contracture was inhibited by IL-10 treatment. In vivo, the results were confirmed by better scar appearance and less collagen accumulation in the IL-10–treated mouse wounds compared with the nontreated controls.[107] In a follow-up study, the authors revealed that IL-10 elicits its antifibrotic effects through the coactivation of the AKT and STAT3 (signal transducer and activator of transcription 3) signaling pathways, and the crosstalk between these pathways blocks the expression of profibrotic genes and the downstream synthesis of ECM components.[28]

This is by far not a complete list of the growth factors/cytokines secreted by ADSCs that potentially may play a role in their antifibrotic actions. Overall, ADSCs modify the wound-healing environment[108] and seem to be protective for fibroblasts[109] via the secretion of a multitude of paracrine factors that act synergistically to achieve this effect.[110,111] The fibroblast is one of the most important cell types governing the tissue repair process. Fibroblasts play a crucial role in wound healing as they secrete components of both the provisional and definitive ECM, take part in granulation tissue formation, and later differentiate into myofibroblasts, which contract the wound and complete tissue remodeling in the final stage of wound healing. Continuous activation of fibroblasts during fibrogenesis leads to the emergence of dysfunctional cells with excessive ECM formation and wound contraction, which are, however, unable to complete the wound-healing process in a physiological and timely fashion.[39] It is suggested that one of the mechanisms of the ADSCs antifibrotic effects is associated with the normalization of fibroblast wound-healing functions.[37,112] The positive effects of ADSCs on fibroblast function were demonstrated in multiple wound healing and antiaging studies and include an enhancement of fibroblast proliferation,[108–111,113] migration,[110,111] and synthetic function[108,110,113] with a concomitant acceleration of wound closure.[110,114] One study demonstrated that earlier wound closure and better scar quality were, however, associated with a lower expression level of α-SMA, suggesting an inhibitory effect of ADSCs on excessive wound contracture,[114] which seems to fall in line with the ADSCs' antifibrotic properties. In addition, ADSCs modulate fibroblast synthetic function by increasing hyaluronic acid fibroblast production.[105,115] Hyaluronic acid, as one of the major components of the ECM, is associated with scarless healing[115] and is thus protective in terms of HTS formation. In a setting of radiation-induced dermal fibrosis, ADSCs seem to protect inherent dermal fibroblasts from radiation damage as evidenced by the preserved cell numbers and a low negative effect of the external irradiation on dermal fibroblast proliferation in ADSC-fibroblast cocultures when compared with fibroblast monocultures.[116]

The other pivotal cell type in the wound-healing process is the keratinocyte, which is the primary cell responsible for re-epithelialization. Crosstalk between fibroblasts and keratinocytes is accomplished through the BM in addition to paracrine signaling and is crucial for normal skin homeostasis and wound healing.[25,117] Disruption of this normal crosstalk is thought to be involved in HTS pathogenesis. For instance, it is suggested that a process known as epithelial-to-mesenchymal transition (EMT) plays an important role here. This phenomenon was originally described as a crucial process

in early embryogenesis in which mesoderm forms from primitive epithelial cells. In adult epithelial tissue, EMT represents the loss of polarity and cell-to-cell adhesions with cytoskeletal rearrangements and a gain of motility in mesenchymal cells.[118] Yan et al[119] performed a histological and immunohistochemical analysis of healing human skin wounds in the phase of re-epithelialization, which showed that keratinocytes in the migrating epithelial tongues at the wound edges acquire mesenchymal markers (vimentin and fibroblast-specific protein 1), suggesting that a mechanism resembling EMT is involved in normal epithelial cell migration and wound closure. However, the mesenchymal markers persisted in the epidermis of HTSs, along with a loss of the epithelial marker E-cadherin, and the EMT-associated transcription factors *slug* and *twist-1* were up-regulated compared with normal skin. In addition, cells in the basal layer of the epidermis seemed detached from the BM, and the BM itself was discontinuous, allowing for penetration of EMT cells into the dermis. The resulting mesenchymal cells, however, lack the properties of normal fibroblasts/myofibroblasts, for instance, α-SMA expression. In cell culture experiments, the authors have found that the EMT changes in keratinocytes were associated with increased levels of TNF-α and TGF-β1, thus suggesting that prolonged inflammation contributes to the ongoing process of EMT in scar epithelium, which in turn produces more dysfunctional fibrotic cells and overall may be one of the key factors in HTS pathogenesis.[119] Similar to the previously described situation with scar fibroblasts, ADSCs have demonstrated positive effects in terms of normalizing keratinocyte function and promoting re-epithelialization.[61,110,113] The positive ADSC effects on keratinocyte proliferation and migration have been linked to the secretion of keratinocyte growth factor[110,117] and PDGF[117] by ADSCs.

Some authors also mention VEGF-mediated stimulation of angiogenesis as a potential antifibrotic mechanism of ADSC/mesenchymal stem cell (MSC) therapies.[37,112] The production of VEGF is a well-known paracrine mechanism of ADSC treatment for various therapeutic purposes.[120–122] An increase in dermal vascularization is undoubtedly one of the favorable effects of fat grafting/ADSC treatment in irradiated fibrotic skin.[32,34] In a setting of developing HTSs, earlier vascularization plays a role in timely wound closure and normal scar formation. However, one must keep in mind that excessive angiogenesis is a characteristic feature of pathological scarring,[123] and therapies targeted on lowering VEGF levels in the wound bed have been developed in order to attenuate HTSs.[124] Bruno et al[13] have, in fact, observed decreased VEGF expression in scar tissue after fat grafting, which correlated with an overall decrease in scar vascular density with a selective improvement of the microvascular pattern at the level of dermal papillae.

Antioxidant Effects of ADSCs

As a result of tissue injury, scar formation/fibrosis is associated with hypoxic conditions in the affected site leading to the generation of reactive oxygen species (ROS). Acute hypoxia is a characteristic feature of injured tissue and is due to the mechanical disruption of microvessel integrity, as well as an increased demand for oxygen by inflammatory and mesenchymal cells migrating to the inflammation site.[125,126] Macrophages are the main source of ROS in the healing wound, which are necessary for the elimination of microbial agents from the wound site.[126] Being an inherent feature of wound healing, hypoxia also plays a role in the pathological patterns of tissue repair, including fibrosis. Fibrotic tissue is characterized by a low microvascular density with decreased oxygen tension. As the result of chronic hypoxia, ROS have potent effects on TGF-β1/Smad activation and downstream collagen accumulation.[127–129]

Currently, a number of studies indicate that ADSCs pose potent antioxidant properties. The protective role of ADSCs in a setting of kidney ischemia-reperfusion injury was confirmed in an animal model and was linked to an up-regulation of antioxidant markers such as glutathione peroxidase (GPx), glutathione reductase, heme oxygenase, NAD(P)H quinone oxidoreductase, and endothelial nitric oxide (NO) synthase, as well as a positive role in enhancing cell survival in the setting of oxidative stress, as confirmed by a down-regulation of the apoptotic marker caspase 3 and an up-regulation of the antiapoptotic marker Bcl-2.[130] In a model of acute lung injury in vitro and in vivo, ADSCs attenuated the severity of the damage by inducing endothelial NO synthase and increasing NO levels in pulmonary microvascular endothelial cells.[131]

Kim et al[132] performed an extensive study of ADSC antioxidant properties by analyzing ADSC-CM. An antioxidant assay revealed that the antioxidant activity of ADSC-CM collected after 72 hours of 4×10^5 ADSC culture in serum-free medium matched that of 100 μM ascorbic acid. A proteomic analysis of ADSC-CM showed the presence of a number of compounds with known antioxidant properties including insulinlike growth factor, insulinlike growth factor–binding proteins, pigment-epithelium–derived factor, placental growth factor, keratinocyte growth factor, PDGF, VEGF, FGF, HGF, latent transforming growth factor β–binding proteins, IL-6, IL-8, and superoxide dismutase (SOD). In a series of in vitro experiments, ADSC-CM demonstrated protective effects on human dermal fibroblasts treated with *tert*-butyl hydroperoxide. For instance, ADSC-CM preserved fibroblast morphology and viability and increased fibroblast survival in this oxidative stress model. When used as a pretreatment regimen 24 hours before the introduction of the oxidative stress, ADSC-CM significantly reduced the degree of apoptosis and activity level of caspase 3 in dermal fibroblasts subjected to *tert*-butyl hydroperoxide. Importantly, ADSC-CM seemed to enhance SOD and GPx activity in fibroblasts.[132] Both SOD and GPx are crucial compounds of the intracellular antioxidant defense mechanism, and their up-regulation may account for enhanced fibroblast survival with ADSC-CM treatment under conditions of oxidative stress. Chae et al[133] explored the antioxidant capacities of protein extracts from ADSC-CM obtained from ADSC culture under hypoxic conditions. The results demonstrated that protein extracts from ADSC-CM exert a reducing power and are effective in scavenging hydrogen peroxide and inhibiting lipid peroxidation. Contrary to the findings by Kim et al,[132] the expression of SOD in dermal fibroblasts decreased with ADSC-CM protein extract treatment. The authors explained this finding by the high antioxidant activity of the ADSC paracrine secretions themselves, which leads to a pronounced reduction of oxidative stress in the surrounding environment and thus makes the additional production of antioxidants by the affected fibroblasts unnecessary.[133] Because MMP dysregulation is linked to tissue damage by ROS,[134] the activity and expression levels of MMPs and TIMPs in dermal fibroblasts were measured after ADSC-CM protein extract treatment. The effects of the ADSC-CM protein extracts on the expression of MMP-1 and MMP-2 and TIMP-1 and TIMP-2 in dermal fibroblasts were ambivalent, depending on the protein concentration and the presence of specific stimulants of MMP expression.[133] Thus, we speculate that ADSCs exhibit regulatory effects on matrix remodeling enzymes, possibly through antioxidant mechanisms, with the net effect depending on the surrounding tissue milieu, and thus might play an important role in orchestrating scar remodeling.

Normalization of the Wound-Healing Process: Direct Differentiation of ADSCs Into Other Cell Types

A large part of ADSC research is currently devoted to the positive wound-healing effects of ADSCs and their potential therapeutic use for healing of chronic wounds. Even though chronic nonhealing wounds and HTSs seem to be on the opposite ends of the wound-healing pathology spectrum, the promotion of normal wound healing could play a positive role in HTS prophylaxis, particularly in wounds prone to prolonged inflammation, with possible resulting dysregulation of the overall tissue regeneration process. However, even established

scars and fibrosis can potentially benefit from the normalization of inherent cell functions by ADSCs, possibly via the same mechanisms that play a role in the ADSC-mediated closure of chronic wounds.

While the paracrine effects of ADSCs on fibroblasts and keratinocytes in terms of wound-healing normalization were discussed earlier in this article, another possible mechanism of ADSC-mediated normalization of the wound-healing process is the direct differentiation of ADSCs into the various cell types necessary for successful and uncomplicated wound healing. Indeed, as observed in multiple experiments, ADSCs have the capacity to differentiate into fibroblasts and keratinocytes.[114,135–137] This mechanism, even though currently not considered to be primary in the ADSC antifibrotic paradigm, nevertheless can certainly account for some of the positive changes observed in fibrotic tissues after fat grafting. However, recent work suggests that ADSC differentiation can also be the source of myofibroblasts that play an ultimate role in fibrogenesis.[58,59,138–140] In collaborative work with our laboratory, Plikus et al[141] demonstrated that mature α-SMA–positive myofibroblasts in the wound bed under certain conditions may redifferentiate into adipocytes. This process requires proximity to a newly regenerated hair follicle, is mediated by the bone morphogenetic protein (BMP), and includes activation of the *Zfp423* embryonic pathway.[141] Contrary to a traditional view of the myofibroblast as a mature terminal cell type, this finding suggests that ADSCs and myofibroblasts in the wound bed may exist in a state of delicate balance, while the introduction of a new external factor (lipoaspirate, new ADSCs, or BMP) shifts the balance toward one of another cell lineage.

Immunomodulatory Effects

When discussing the crucial role of wound-healing normalization in combating dermal fibrosis, the picture would be incomplete without touching on the importance of inflammation and immune effector cells in its pathogenesis. Prolonged inflammation with a persistence of immune cells in the wound bed and increased levels of proinflammatory cytokines are directly connected to exaggerated wound-healing responses and the formation of inappropriate scars. As an example, the role of persisting TNF-α in promoting EMT as an important mechanism of wound-healing dysregulation in HTSs was discussed in the previous section. Studies show that ADSCs are capable of decreasing the inflammatory response in healing wounds.[13,40,109,110]

Carceller et al[142] demonstrated the anti-inflammatory effect of ADSCs in an in vivo mouse model. According to their work, both ADSCs and ADSC-CM effectively suppressed the inflammatory response by down-regulating leukotriene B4 and other chemotactic chemokines, which thus inhibited early leukocyte migration. The experiment also showed a significant reduction of important inflammatory mediators such as IL-1β, IL-6, TNF-α, and TNF-stimulated gene 6. These effects were in part related to the inhibition of the nuclear factor κB pathway.[142]

In the later stages of inflammation, ADSCs play an important role in regulating macrophage function. Currently, 2 distinct macrophage phenotypes are recognized by scientists—the classic proinflammatory M1 and the alternatively activated anti-inflammatory M2 phenotype.[143] The M1 phenotype is capable of producing high levels of inflammatory cytokines and thus prolonging inflammation and potentially causing adverse outcomes on wound healing if overexpressed. A number of studies confirmed the capacity of ADSCs to promote a phenotype switch in macrophages, which favors the anti-inflammatory M2 phenotype over the proinflammatory M1.[143–145]

Infiltration with antigen-presenting cells, mast cells, and T cells is a characteristic feature of the later stages of HTS pathogenesis.[146] Bruno et al[13] observed a decrease in Langerhans cell density in scar tissue after fat injection. Yun et al[40] linked the positive results of ADSC scar treatment to a reduced number of mast cells in histological samples from the treatment group. Mast cells are thought to have direct effects on stimulating the proliferation of fibroblasts, their differentiation into myofibroblasts, ECM synthesis, and excessive wound contraction.[147] Furthermore, ADSCs possess strong immunosuppressive properties by suppressing T-cell responses; for instance, their addition suppresses mixed lymphocyte reactions.[148,149] Adipose-derived stem cells secrete paracrine factors with known negative effects on T-cell activation, such as indoleamine 2,3 dioxygenase, prostaglandin E_2, cyclooxygenase 2, and TGF-β1.[37,148–153]

Thus, ADSCs cause potent modulatory effects on different elements of the wound-healing cascade during scar formation. They are capable of suppressing inflammatory mediator secretion, promoting anti-inflammatory macrophage functions, and reducing the numbers of antigen-presenting and mast cells, as well as regulating T-cell responses.

The p53-Dependent Mechanism

When exploring the consequences of fat graft treatment on postburn scars, Bruno et al[13] have noted that p53 expression was significantly reduced in the untreated samples with a concomitant increased activity of p63. This finding was speculated to be an important feature of HTS pathogenesis as the antiapoptotic gene p63 putatively produces a sustained diffuse proliferative, differentiative, and regenerative activity. However, the ratio was revised after treatment, with an increase in proapoptotic p53 activity predominantly in the basal layer of the epidermis.[13] This finding was followed with a study by Liu et al,[154] in which the authors specifically explored the role of the p53 gene on the antifibrotic effects of MSCs in an HTS rabbit ear model.

The tumor-suppressor gene p53 has been implicated in the pathogenesis of pathological scar formation including HTSs.[155] Liu et al[154] explored whether its expression is somehow linked to the positive antifibrotic effects of MSC administration, by using both wild-type and p53-knockdown MSCs for treatment of HTSs in a rabbit ear model. Wild-type MSCs reduced unfavorable scarring, showing less collagen deposition, reduced vascularity, and less TβRI and α-SMA expression in the forming scar tissue, suggesting less fibroblast-to-myofibroblast differentiation. However, p53-deficient MSCs had little influence on the development of HTSs. Moreover, MSCs with a p53 gene knockdown promoted the proliferation of fibroblasts by increasing NO production. These results suggest that p53 is involved in the MSC-mediated antifibrotic effect by down-regulating fibroblast-to-myofibroblast differentiation and inhibiting NO-mediated fibroblast proliferation.[154]

While p53 activity and the normal p53/p63 ratio might be disrupted in HTSs, the introduction of ADSCs with a normally functioning proapoptotic p53 gene seems to restore this ratio, which leads to a decreased cell proliferation and scar reduction.

Inhibition of the p38/MAPK Signaling Pathway

Finally, a recently published study by Li et al[156] unravels one of the earlier unexplored molecular mechanisms involved in ADSC-mediated suppression of fibrosis. The authors studied the role of the p38/MAPK signaling pathway in this therapeutic setting, as p38/MAPK is one of the molecular mechanisms involved in fibrogenesis.[157–159] Cultures of human scar fibroblasts, ex vivo–cultured HTS tissues, and mouse excisional wounds were treated with ADSC-CM. This treatment, as expected, decreased the expression of collagen types I and II and α-SMA. It also resulted in a faster rate of wound closure in the mice and a more organized collagen pattern with thinner fibers in the scar tissue cultures. In addition, ADSC-CM down-regulated the protein level of phospho-p38 in treated fibroblasts. Similar to these results, treatment of fibroblasts and forming scars with a specific p38 inhibitor resulted in an obvious decline in the expression of collagen types I and II and α-SMA, with a more organized arrangement of collagen fibers in the scar tissues. On the other hand, treatment with anisomycin, a p38 agonist, up-regulated fibrotic proteins and resulted in a more random collagen distribution pattern. Overall, these

results demonstrate that switching the p38/MAPK signaling pathway on and off had a pronounced impact on fibroblast synthetic function and the collagen distribution pattern of forming and established scars. Thus, the authors suggested that inhibition of the p38/MAPK signaling pathway is at least one of the underlying mechanisms of the ADSC-mediated antiscarring effect.[156]

The Role of Direct Cell-to-Cell Communication

It is readily apparent from the findings noted previously that secreted factors seem to play a major role in ADSC-mediated scar resolution, with the mechanism of action primarily attributed to the paracrine antifibrotic actions of the ADSCs. However, some publications suggest that direct cell-to-cell contact of ADSCs with cells in the scar tissue plays a potent role in fibrosis reduction. Zhang et al[37] demonstrated reduced expression of α-SMA and collagen type I in scar biopsies after intralesional injection of both ADSCs and ADSC-CM in a rabbit ear HTS model, with absolute values of α-SMA and collagen type I mRNA being lower in ADSC therapy versus ADSC-CM. The scar elevation index, an objective measurement of the degree of scar hypertrophy, was also lower with the ADSC injection compared with the cell-free treatment, and a fluorescent tracking assay confirmed that a large number of ADSCs were alive in the area 3 weeks after the cell injection. These results suggest that ADSCs remain viable postinjection and that their increased therapeutic efficacy compared with ADSC-CM is possibly due to the fact that not only paracrine mechanisms, but also direct cell-to-cell interactions, play a role in ADSC-mediated antifibrotic actions.[37]

Another study by Verhoekx et al[31] investigated the in vitro interactions of myofibroblasts from Dupuytren disease with ADSCs. When added to Dupuytren myofibroblast monoculture, conditioned medium from both indirect and direct ADSC-myofibroblast cocultures decreased myofibroblast proliferation and numbers. However, this decrease was significantly more evident with the conditioned medium from the direct cocultures. This suggests that the release of paracrine antifibrotic factors from the ADSCs is possibly up-regulated by a cell-to-cell contact-dependent signaling mechanism; for example, ADSC contact with myofibroblasts leads to the secretion of compounds that in turn inhibit myofibroblast proliferation and reduce the recruitment of additional myofibroblasts.[31]

Future Directions

Although many mechanisms of action of ADSCs in the setting of fibrosis have been discussed in this review, a comprehensive description of the ADSC antifibrotic mechanism is still lacking. Successful treatment of HTSs and other fibrotic conditions with transplanted fat or ADSCs is to date confined to anecdotal cases. A thorough understanding of the underlying antifibrotic mechanisms will help us develop protocols for more successful treatments. For instance, we still do not know if heterogenous fat grafting or homogenous ADSC administration to healing wounds with a high risk of fibrosis development is as safe and effective as an injection to established HTSs.[39] The difference between developing and established HTSs in terms of the activity of profibrotic and antifibrotic pathways and the way in which these may be influenced by fat or ADSC injection has to be studied in greater detail. This way, we can get a better understanding of how fat grafts or ADSCs can be introduced to developing scars in a more timely fashion in order to get the best clinical results. The enrichment of the transplanted fat graft with certain paracrine factors with known antifibrotic activity (eg, TGF-β3, BMP, HGF, bFGF, etc) may be another avenue for further research and clinical trials. The fact that transformation or dedifferentiation of myofibroblasts to adipocytes may take place under the influence of MSCs and BMP/*Zfp423* mediation presents a real possibility of a fat-based therapeutic being used in established scars.[141] This is an exciting new avenue for exploration and one that our laboratory is actively pursuing.

CONCLUSIONS

The encouraging results of fat grafting introduced into exaggerated scars and sites of dermal fibrosis seem to be mediated by a variety of molecular mechanisms. Adipose-derived stem cells in the transplanted fat tissue secrete multiple paracrine factors, which pose potent anti-inflammatory, immunomodulatory, antioxidant, and antifibrotic effects. Normalization of fibroblast and keratinocyte function and direct differentiation of ADSCs to various cell types necessary for normal wound healing also play an important role. Further studies are needed to provide a deeper understanding of the difference between established and developing HTSs in terms of their treatment with ADSCs/fat injections.

REFERENCES

1. Coleman SR. Long-term survival of fat transplants: controlled demonstrations. *Aesthetic Plast Surg*. 1995;19:421–425.
2. Coleman SR. Structural fat grafts: the ideal filler? *Clin Plast Surg*. 2001;28:111–119.
3. Rigotti G, Marchi A, Galiè M, et al. Clinical treatment of radiotherapy tissue damage by lipoaspirate transplant: a healing process mediated by adipose-derived adult stem cells. *Plast Reconstr Surg*. 2007;119:1409–1422; discussion 1423–4.
4. Caviggioli F, Klinger F, Villani F, et al. Correction of cicatricial ectropion by autologous fat graft. *Aesthetic Plast Surg*. 2008;32:555–557.
5. Klinger M, Marazzi M, Vigo D, et al. Fat injection for cases of severe burn outcomes: a new perspective of scar remodeling and reduction. *Aesthetic Plast Surg*. 2008;32:465–469.
6. Phulpin B, Gangloff P, Tran N, et al. Rehabilitation of irradiated head and neck tissues by autologous fat transplantation. *Plast Reconstr Surg*. 2009;123:1187–1197.
7. Damgaard OE, Siemssen PA. Lipografted tenolysis. *J Plast Reconstr Aesthet Surg*. 2010;63:e637–e638.
8. Serra-Renom JM, Muñoz-Olmo JL, Serra-Mestre JM. Fat grafting in post-mastectomy breast reconstruction with expanders and prostheses in patients who have received radiotherapy: formation of new subcutaneous tissue. *Plast Reconstr Surg*. 2010;125:12–18.
9. Hovius SE, Kan HJ, Smit X, et al. Extensive percutaneous aponeurotomy and lipografting: a new treatment for Dupuytren disease. *Plast Reconstr Surg*. 2011;128:221–228.
10. Brongo S, Nicoletti GF, La Padula S, et al. Use of lipofilling for the treatment of severe burn outcomes. *Plast Reconstr Surg*. 2012;130:374e–376e.
11. Caviggioli F, Forcellini D, Vinci V, et al. Employment of needles: a different technique for fat placement. *Plast Reconstr Surg*. 2012;130:373e–374e.
12. Ulrich D, Ulrich F, van Doorn L, et al. Lipofilling of perineal and vaginal scars: a new method for improvement of pain after episiotomy and perineal laceration. *Plast Reconstr Surg*. 2012;129:593e–594e.
13. Bruno A, Delli Santi G, Fasciani L, et al. Burn scar lipofilling: immunohistochemical and clinical outcomes. *J Craniofac Surg*. 2013;24:1806–1814.
14. Khouri RK, Smit JM, Cardoso E, et al. Percutaneous aponeurotomy and lipofilling: a regenerative alternative to flap reconstruction? *Plast Reconstr Surg*. 2013;132:1280–1290.
15. Klinger M, Caviggioli F, Klinger FM, et al. Autologous fat graft in scar treatment. *J Craniofac Surg*. 2013;24:1610–1615.
16. Mazzola IC, Cantarella G, Mazzola RF. Management of tracheostomy scar by autologous fat transplantation: a minimally invasive new approach. *J Craniofac Surg*. 2013;24:1361–1364.
17. Maione L, Memeo A, Pedretti L, et al. Autologous fat graft as treatment of post short stature surgical correction scars. *Injury*. 2014;45:S126–S132.
18. Maione L, Vinci V, Caviggioli F, et al. Autologous fat graft in postmastectomy pain syndrome following breast conservative surgery and radiotherapy. *Aesthetic Plast Surg*. 2014;38:528–532.
19. Pallua N, Baroncini A, Alharbi Z, et al. Improvement of facial scar appearance and microcirculation by autologous lipofilling. *J Plast Reconstr Aesthet Surg*. 2014;67:1033–1037.
20. Del Papa N, Caviggioli F, Sambataro D, et al. Autologous fat grafting in the treatment of fibrotic perioral changes in patients with systemic sclerosis. *Cell Transplant*. 2015;24:63–72.
21. Krzesniak NE, Noszczyk BH. Autologous fat transfer in secondary carpal tunnel release. *Plast Reconstr Surg Glob Open*. 2015;3:e401.
22. Piccolo NS, Piccolo MS, Piccolo MT. Fat grafting for treatment of burns, burn scars, and other difficult wounds. *Clin Plast Surg*. 2015;42:263–283.

23. Ding J, Tredget EE. The role of chemokines in fibrotic wound healing. *Adv Wound Care (New Rochelle)*. 2015;4:673–686.
24. Artlett CM. Inflammasomes in wound healing and fibrosis. *J Pathol*. 2013;229: 157–167.
25. Yang SW, Geng ZJ, Ma K, et al. Comparison of the histological morphology between normal skin and scar tissue. *J Huazhong Univ Sci Technolog Med Sci*. 2016;36:265–269.
26. Procter F. Rehabilitation of the burn patient. *Indian J Plast Surg*. 2010;43(suppl): S101–S113.
27. Wynn TA. Integrating mechanisms of pulmonary fibrosis. *J Exp Med*. 2011;208: 1339–1350.
28. Shi J, Li J, Guan H, et al. Anti-fibrotic actions of interleukin-10 against hypertrophic scarring by activation of PI3K/AKT and STAT3 signaling pathways in scar-forming fibroblasts. *PLoS One*. 2014;9:e98228.
29. Breitkreutz D, Mirancea N, Nischt R. Basement membranes in skin: unique matrix structures with diverse functions? *Histochem Cell Biol*. 2009;132:1–10.
30. Ko MS, Marinkovich MP. Role of dermal-epidermal basement membrane zone in skin, cancer, and developmental disorders. *Dermatol Clin*. 2010;28:1–16.
31. Verhoekx JS, Mudera V, Walbeehm ET, et al. Adipose-derived stem cells inhibit the contractile myofibroblast in Dupuytren's disease. *Plast Reconstr Surg*. 2013; 132:1139–1148.
32. Luan A, Duscher D, Whittam AJ, et al. Cell-assisted lipotransfer improves volume retention in irradiated recipient sites and rescues radiation-induced skin changes. *Stem Cells*. 2016;34:668–673.
33. Sultan SM, Stern CS, Allen RJ Jr, et al. Human fat grafting alleviates radiation skin damage in a murine model. *Plast Reconstr Surg*. 2011;128:363–372.
34. Garza RM, Paik KJ, Chung MT, et al. Studies in fat grafting: part III. Fat grafting irradiated tissue–improved skin quality and decreased fat graft retention. *Plast Reconstr Surg*. 2014;134:249–257.
35. Gumucio JP, Flood MD, Roche SM, et al. Stromal vascular stem cell treatment decreases muscle fibrosis following chronic rotator cuff tear. *Int Orthop*. 2016; 40:759–764.
36. Sun W, Ni X, Sun S, et al. Adipose-derived stem cells alleviate radiation-induced muscular fibrosis by suppressing the expression of TGF-β1. *Stem Cells Int*. 2016;2016:5638204.
37. Zhang Q, Liu LN, Yong Q, et al. Intralesional injection of adipose-derived stem cells reduces hypertrophic scarring in a rabbit ear model. *Stem Cell Res Ther*. 2015;6:145.
38. Finnson KW, McLean S, Di Guglielmo GM, et al. Dynamics of transforming growth factor beta signaling in wound healing and scarring. *Adv Wound Care (New Rochelle)*. 2013;2:195–214.
39. Chiang RS, Borovikova AA, King K, et al. Current concepts related to hypertrophic scarring in burn injuries. *Wound Repair Regen*. 2016;24:466–477.
40. Yun IS, Jeon YR, Lee WJ, et al. Effect of human adipose derived stem cells on scar formation and remodeling in a pig model: a pilot study. *Dermatol Surg*. 2012;38:1678–1688.
41. Jiang X, Jiang X, Qu C, et al. Intravenous delivery of adipose-derived mesenchymal stromal cells attenuates acute radiation-induced lung injury in rats. *Cytotherapy*. 2015;17:560–570.
42. Yu FX, Su LF, Dai CL, et al. Inhibition of pancreatic stellate cell activity by adipose-derived stem cells. *Hepatobiliary Pancreat Dis Int*. 2015;14:215–221.
43. Chen L, Wang DL, Wei ZR, et al. Effects of local transplantation of autologous adipose-derived mesenchymal stem cells on the formation of hyperplastic scar on rabbit ears [in Chinese]. *Zhonghua Shao Shang Za Zhi*. 2016;32:582–587.
44. Hiwatashi N, Bing R, Kraja I, et al. Mesenchymal stem cells have antifibrotic effects on transforming growth factor-β1–stimulated vocal fold fibroblasts. *Laryngoscope*. 2017;127:E35–E41.
45. Wrana JL, Attisano L, Wieser R, et al. Mechanism of activation of the TGF-beta receptor. *Nature*. 1994;370:341–347.
46. Peng C. The TGF-beta superfamily and its roles in the human ovary and placenta. *J Obstet Gynaecol Can*. 2003;25:834–844.
47. Mulloy B, Rider CC. The bone morphogenetic proteins and their antagonists. *Vitam Horm*. 2015;99:63–90.
48. Davis H, Raja E, Miyazono K, et al. Mechanisms of action of bone morphogenetic proteins in cancer. *Cytokine Growth Factor Rev*. 2016;27:81–92.
49. Olguin-Alor R, de la Fuente-Granada M, Bonifaz LC, et al. A key role for inhibins in dendritic cell maturation and function. *PLoS One*. 2016;11:e0167813.
50. Kane CJ, Hebda PA, Mansbridge JN, et al. Direct evidence for spatial and temporal regulation of transforming growth factor beta 1 expression during cutaneous wound healing. *J Cell Physiol*. 1991;148:157–173.
51. Lichtman MK, Otero-Vinas M, Falanga V. Transforming growth factor beta (TGF-β) isoforms in wound healing and fibrosis. *Wound Repair Regen*. 2016; 24:215–222.
52. Levine JH, Moses HL, Gold LI, et al. Spatial and temporal patterns of immunoreactive transforming growth factor beta 1, beta 2, and beta 3 during excisional wound repair. *Am J Pathol*. 1993;143:368–380.
53. Wahl S, *Cytokines in Wound Healing*. R&D Systems, Inc; 2002. Available at: http://www.rndsystems.com/mini_review_detail_objectname_MR02_CytokineWoundHealing.aspx. Accessed October 9, 2017.
54. Spiekman M, Przybyt E, Plantinga JA, et al. Adipose tissue–derived stromal cells inhibit TGF-β1–induced differentiation of human dermal fibroblasts and keloid scar–derived fibroblasts in a paracrine fashion. *Plast Reconstr Surg*. 2014;134: 699–712.
55. Occleston NL, O'Kane S, Laverty HG, et al. Discovery and development of avotermin (recombinant human transforming growth factor beta 3): a new class of prophylactic therapeutic for the improvement of scarring. *Wound Repair Regen*. 2011;19 suppl 1:s38–s48.
56. Huang S, Wu Y, Gao D, et al. Paracrine action of mesenchymal stromal cells delivered by microspheres contributes to cutaneous wound healing and prevents scar formation in mice. *Cytotherapy*. 2015;17:922–931.
57. Wu Y, Peng Y, Gao D, et al. Mesenchymal stem cells suppress fibroblast proliferation and reduce skin fibrosis through a TGF-β3–dependent activation. *Int J Low Extrem Wounds*. 2015;14:50–62.
58. Desai VD, Hsia HC, Schwarzbauer JE. Reversible modulation of myofibroblast differentiation in adipose-derived mesenchymal stem cells. *PLoS One*. 2014; 9:e86865.
59. Kakudo N, Kushida S, Suzuki K, et al. Effects of transforming growth factor-beta1 on cell motility, collagen gel contraction, myofibroblastic differentiation, and extracellular matrix expression of human adipose-derived stem cell. *Hum Cell*. 2012;25:87–95.
60. Cho JW, Kang MC, Lee KS. TGF-β1–treated ADSCs-CM promotes expression of type I collagen and MMP-1, migration of human skin fibroblasts, and wound healing in vitro and in vivo. *Int J Mol Med*. 2010;26:901–906.
61. Moon KM, Park YH, Lee JS, et al. The effect of secretory factors of adipose-derived stem cells on human keratinocytes. *Int J Mol Sci*. 2012;13:1239–1257.
62. Banyard DA, Salibian AA, Widgerow AD, et al. Implications for human adipose-derived stem cells in plastic surgery. *J Cell Mol Med*. 2015;19:21–30.
63. Bajek A, Gurtowska N, Olkowska J, et al. Adipose-derived stem cells as a tool in cell-based therapies. *Arch Immunol Ther Exp (Warsz)*. 2016;64:443–454.
64. Suga H, Eto H, Shigeura T, et al. IFATS collection: fibroblast growth factor-2–induced hepatocyte growth factor secretion by adipose-derived stromal cells inhibits postinjury fibrogenesis through a c-Jun N-terminal kinase–dependent mechanism. *Stem Cells*. 2009;27:238–249.
65. Banyard DA, Sarantopoulos CN, Borovikova AA, et al. Phenotypic analysis of stromal vascular fraction after mechanical shear reveals stress-induced progenitor populations. *Plast Reconstr Surg*. 2016;138:237e–247e.
66. Aiba-Kojima E, Tsuno NH, Inoue K, et al. Characterization of wound drainage fluids as a source of soluble factors associated with wound healing: comparison with platelet-rich plasma and potential use in cell culture. *Wound Repair Regen*. 2007;15:511–520.
67. Chen JC, Lin BB, Hu HW, et al. NGF accelerates cutaneous wound healing by promoting the migration of dermal fibroblasts via the PI3K/Akt-Rac1-JNK and ERK pathways. *Biomed Res Int*. 2014;2014:547187.
68. Zhang M, Sun L, Wang X, et al. Activin B promotes BMSC-mediated cutaneous wound healing by regulating cell migration via the JNK-ERK signaling pathway. *Cell Transplant*. 2014;23:1061–1073.
69. Choi YH, Yang DJ, Kulkarni A, et al. Mycosporine-like amino acids promote wound healing through focal adhesion kinase (FAK) and mitogen-activated protein kinases (MAP kinases) signaling pathway in keratinocytes. *Mar Drugs*. 2015;13:7055–7066.
70. Ono I. The effects of basic fibroblast growth factor (bFGF) on the breaking strength of acute incisional wounds. *J Dermatol Sci*. 2002;29:104–113.
71. Lohmeyer JA, Liu F, Krüger S, et al. Use of gene-modified keratinocytes and fibroblasts to enhance regeneration in a full skin defect. *Langenbecks Arch Surg*. 2011;396:543–550.
72. Abe M, Yokoyama Y, Ishikawa O. A possible mechanism of basic fibroblast growth factor-promoted scarless wound healing: the induction of myofibroblast apoptosis. *Eur J Dermatol*. 2012;22:46–53.
73. Shi HX, Lin C, Lin BB, et al. The anti-scar effects of basic fibroblast growth factor on the wound repair in vitro and in vivo. *PLoS One*. 2013;8:e59966.
74. Funato N, Moriyama K, Shimokawa H, et al. Basic fibroblast growth factor induces apoptosis in myofibroblastic cells isolated from rat palatal mucosa. *Biochem Biophys Res Commun*. 1997;240:21–26.
75. Kawai-Kowase K, Sato H, Oyama Y, et al. Basic fibroblast growth factor antagonizes transforming growth factor-beta1–induced smooth muscle gene expression through extracellular signal–regulated kinase 1/2 signaling pathway activation. *Arterioscler Thromb Vasc Biol*. 2004;24:1384–1390.

76. Xie JL, Bian HN, Qi SH, et al. Basic fibroblast growth factor (bFGF) alleviates the scar of the rabbit ear model in wound healing. *Wound Repair Regen*. 2008; 16:576–581.
77. Xie J, Bian H, Qi S, et al. Effects of basic fibroblast growth factor on the expression of extracellular matrix and matrix metalloproteinase-1 in wound healing. *Clin Exp Dermatol*. 2008;33:176–182.
78. Kamada Y, Yoshida Y, Saji Y, et al. Transplantation of basic fibroblast growth factor-pretreated adipose tissue–derived stromal cells enhances regression of liver fibrosis in mice. *Am J Physiol Gastrointest Liver Physiol*. 2009;296: G157–G167.
79. Tang WP, Akahoshi T, Piao JS, et al. Basic fibroblast growth factor–treated adipose tissue–derived mesenchymal stem cell infusion to ameliorate liver cirrhosis via paracrine hepatocyte growth factor. *J Gastroenterol Hepatol*. 2015. 30: 1065–1074.
80. Horikoshi-Ishihara H, Tobita M, Tajima S, et al. Coadministration of adipose-derived stem cells and control-released basic fibroblast growth factor facilitates angiogenesis in a murine ischemic hind limb model. *J Vasc Surg*. 2016;64: 1825–1834 e1.
81. Khan S, Villalobos MA, Choron RL, et al. Fibroblast growth factor and vascular endothelial growth factor play a critical role in endotheliogenesis from human adipose-derived stem cells. *J Vasc Surg*. 2017;65:1483–1492.
82. Wang B, Ma X, Zhao L, et al. Injection of basic fibroblast growth factor together with adipose-derived stem cell transplantation: improved cardiac remodeling and function in myocardial infarction. *Clin Exp Med*. 2016;16:539–550.
83. Lu F, Zhao X, Wu J, et al. MSCs transfected with hepatocyte growth factor or vascular endothelial growth factor improve cardiac function in the infarcted porcine heart by increasing angiogenesis and reducing fibrosis. *Int J Cardiol*. 2013; 167:2524–2532.
84. Chen H, Xia R, Li Z, et al. Mesenchymal stem cells combined with hepatocyte growth factor therapy for attenuating ischaemic myocardial fibrosis: assessment using multimodal molecular imaging. *Sci Rep*. 2016;6:33700.
85. Gazdhar A, Grad I, Tamò L, et al. The secretome of induced pluripotent stem cells reduces lung fibrosis in part by hepatocyte growth factor. *Stem Cell Res Ther*. 2014;5:123.
86. Dong LH, Jiang YY, Liu YJ, et al. The anti-fibrotic effects of mesenchymal stem cells on irradiated lungs via stimulating endogenous secretion of HGF and PGE_2. *Sci Rep*. 2015;5:8713.
87. Cahill EF, Kennelly H, Carty F, et al. Hepatocyte growth factor is required for mesenchymal stromal cell protection against bleomycin-induced pulmonary fibrosis. *Stem Cells Transl Med*. 2016;5:1307–1318.
88. Lan YW, Theng SM, Huang TT, et al. Oncostatin M-preconditioned mesenchymal stem cells alleviate bleomycin-induced pulmonary fibrosis through paracrine effects of the hepatocyte growth factor. *Stem Cells Transl Med*. 2016.
89. Xia Y, Xia YF, Lv Q, et al. Madecassoside ameliorates bleomycin-induced pulmonary fibrosis in mice through promoting the generation of hepatocyte growth factor via PPAR-γ in colon. *Br J Pharmacol*. 2016;173:1219–1235.
90. Ogaly HA, Eltablawy NA, El-Behairy AM, et al. Hepatocyte growth factor mediates the antifibrogenic action of *Ocimum bacilicum* essential oil against CCL4-induced liver fibrosis in rats. *Molecules*. 2015;20:13518–13535.
91. Mohamed HE, Elswefy SE, Rashed LA, et al. Bone marrow–derived mesenchymal stem cells effectively regenerate fibrotic liver in bile duct ligation rat model. *Exp Biol Med (Maywood)*. 2016;241:581–591.
92. Gong R, Rifai A, Tolbert EM, et al. Hepatocyte growth factor ameliorates renal interstitial inflammation in rat remnant kidney by modulating tubular expression of macrophage chemoattractant protein-1 and RANTES. *J Am Soc Nephrol*. 2004;15:2868–2881.
93. Liu Y. Hepatocyte growth factor in kidney fibrosis: therapeutic potential and mechanisms of action. *Am J Physiol Renal Physiol*. 2004;287:F7–F16.
94. Herrero-Fresneda I, Torras J, Franquesa M, et al. HGF gene therapy attenuates renal allograft scarring by preventing the profibrotic inflammatory-induced mechanisms. *Kidney Int*. 2006;70:265–274.
95. Liu Y, Yang J. Hepatocyte growth factor: new arsenal in the fights against renal fibrosis? *Kidney Int*. 2006;70:238–240.
96. Iekushi K, Taniyama Y, Azuma J, et al. Hepatocyte growth factor attenuates renal fibrosis through TGF-β1 suppression by apoptosis of myofibroblasts. *J Hypertens*. 2010;28:2454–2461.
97. Stewart N, Chade AR. Renoprotective effects of hepatocyte growth factor in the stenotic kidney. *Am J Physiol Renal Physiol*. 2013;304:F625–F633.
98. Hirano S, Bless DM, Rousseau B, et al. Prevention of vocal fold scarring by topical injection of hepatocyte growth factor in a rabbit model. *Laryngoscope*. 2004; 114:548–556.
99. Inagaki Y, Higashi K, Kushida M, et al. Hepatocyte growth factor suppresses profibrogenic signal transduction via nuclear export of Smad3 with galectin-7. *Gastroenterology*. 2008;134:1180–1190.
100. Faehling M, Hetzel M, Anders D, et al. Antifibrotic role of HGF in sarcoidosis. *Lung*. 2012;190:303–312.
101. Sherriff-Tadano R, Ohta A, Morito F, et al. Antifibrotic effects of hepatocyte growth factor on scleroderma fibroblasts and analysis of its mechanism. *Mod Rheumatol*. 2006;16:364–371.
102. Min JK, Lee YM, Kim JH, et al. Hepatocyte growth factor suppresses vascular endothelial growth factor-induced expression of endothelial ICAM-1 and VCAM-1 by inhibiting the nuclear factor-kappaB pathway. *Circ Res*. 2005;96: 300–307.
103. Hayden MS, West AP, Ghosh S. NF-kappaB and the immune response. *Oncogene*. 2006;25:6758–6780.
104. Asano Y, Iimuro Y, Son G, et al. Hepatocyte growth factor promotes remodeling of murine liver fibrosis, accelerating recruitment of bone marrow–derived cells into the liver. *Hepatol Res*. 2007;37:1080–1094.
105. Kumai Y, Kobler JB, Park H, et al. Modulation of vocal fold scar fibroblasts by adipose-derived stem/stromal cells. *Laryngoscope*. 2010;120:330–337.
106. Nakagome K, Dohi M, Okunishi K, et al. In vivo IL-10 gene delivery attenuates bleomycin induced pulmonary fibrosis by inhibiting the production and activation of TGF-beta in the lung. *Thorax*. 2006;61:886–894.
107. Shi JH, Guan H, Shi S, et al. Protection against TGF-β1–induced fibrosis effects of IL-10 on dermal fibroblasts and its potential therapeutics for the reduction of skin scarring. *Arch Dermatol Res*. 2013;305:341–352.
108. Shen X, Du Y, Shen W, et al. Adipose-derived stem cells promote human dermal fibroblast function and increase senescence-associated β-galactosidase mRNA expression through paracrine effects. *Mol Med Rep*, 2014;10:3068–3072.
109. Atalay S, Coruh A, Deniz K. Stromal vascular fraction improves deep partial thickness burn wound healing. *Burns*. 2014;40:1375–1383.
110. Kim WS, Park BS, Sung JH, et al. Wound healing effect of adipose-derived stem cells: a critical role of secretory factors on human dermal fibroblasts. *J Dermatol Sci*. 2007;48:15–24.
111. Zhao J, Hu L, Liu J, et al. The effects of cytokines in adipose stem cell–conditioned medium on the migration and proliferation of skin fibroblasts in vitro. *Biomed Res Int*. 2013;2013:578479.
112. Jackson WM, Nesti LJ, Tuan RS. Mesenchymal stem cell therapy for attenuation of scar formation during wound healing. *Stem Cell Res Ther*. 2012;3:20.
113. Lee SH, Jin SY, Song JS, et al. Paracrine effects of adipose-derived stem cells on keratinocytes and dermal fibroblasts. *Ann Dermatol*. 2012;24:136–143.
114. Uysal CA, Tobita M, Hyakusoku H, et al. The effect of bone-marrow–derived stem cells and adipose-derived stem cells on wound contraction and epithelization. *Adv Wound Care (New Rochelle)*. 2014;3:405–413.
115. Jung H, Kim HH, Lee DH, et al. Transforming growth factor-beta 1 in adipose derived stem cells conditioned medium is a dominant paracrine mediator determines hyaluronic acid and collagen expression profile. *Cytotechnology*. 2011; 63:57–66.
116. Haubner F, Muschter D, Pohl F, et al. A co-culture model of fibroblasts and adipose tissue–derived stem cells reveals new insights into impaired wound healing after radiotherapy. *Int J Mol Sci*. 2015;16:25947–25958.
117. Alexaki VI, Simantiraki D, Panayiotopoulou M, et al. Adipose tissue–derived mesenchymal cells support skin reepithelialization through secretion of KGF-1 and PDGF-BB: comparison with dermal fibroblasts. *Cell Transplant*. 2012;21: 2441–2454.
118. Levayer R, Lecuit T. Breaking down EMT. *Nat Cell Biol*. 2008;10:757–759.
119. Yan C, Grimm WA, Garner WL, et al. Epithelial to mesenchymal transition in human skin wound healing is induced by tumor necrosis factor-alpha through bone morphogenic protein-2. *Am J Pathol*. 2010;176:2247–2258.
120. Salgado AJ, Reis RL, Sousa NJ, et al. Adipose tissue derived stem cells secretome: soluble factors and their roles in regenerative medicine. *Curr Stem Cell Res Ther*. 2010;5:103–110.
121. Sheng L, Yang M, Liang Y, et al. Adipose tissue–derived stem cells (ADSCs) transplantation promotes regeneration of expanded skin using a tissue expansion model. *Wound Repair Regen*. 2013;21:746–754.
122. Yamada S, Shimada M, Utsunomiya T, et al. Trophic effect of adipose tissue–derived stem cells on porcine islet cells. *J Surg Res*. 2014;187:667–672.
123. van der Veer WM, Niessen FB, Ferreira JA, et al. Time course of the angiogenic response during normotrophic and hypertrophic scar formation in humans. *Wound Repair Regen*. 2011;19:292–301.
124. Diao JS, Xia WS, Guo SZ. Bevacizumab: a potential agent for prevention and treatment of hypertrophic scar. *Burns*. 2010;36:1136–1137.
125. Lokmic Z, Musyoka J, Hewitson TD, et al. Hypoxia and hypoxia signaling in tissue repair and fibrosis. *Int Rev Cell Mol Biol*. 2012;296:139–185.
126. Ruthenborg RJ, Ban JJ, Wazir A, et al. Regulation of wound healing and fibrosis by hypoxia and hypoxia-inducible factor-1. *Mol Cells*. 2014;37:637–643.

127. Lijnen PJ, van Pelt JF, Fagard RH. Stimulation of reactive oxygen species and collagen synthesis by angiotensin II in cardiac fibroblasts. *Cardiovasc Ther*. 2012;30:e1–e8.
128. Park IH, Park SJ, Cho JS, et al. Role of reactive oxygen species in transforming growth factor beta1–induced alpha smooth-muscle actin and collagen production in nasal polyp-derived fibroblasts. *Int Arch Allergy Immunol*. 2012;159: 278–286.
129. Zhao L, Wei Z, Yang F, et al. Transforming growth factor-β1 induced cellular proliferation and collagen synthesis was mediated by reactive oxygen species in pulmonary fibroblasts. *Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi*. 2015;33:15–19.
130. Chen YT, Sun CK, Lin YC, et al. Adipose-derived mesenchymal stem cell protects kidneys against ischemia-reperfusion injury through suppressing oxidative stress and inflammatory reaction. *J Transl Med*. 2011;9:51.
131. Gao P, Yang X, Mungur L, et al. Adipose tissue–derived stem cells attenuate acute lung injury through eNOS and eNOS-derived NO. *Int J Mol Med*. 2013; 31:1313–1318.
132. Kim WS, Park BS, Kim HK, et al. Evidence supporting antioxidant action of adipose-derived stem cells: protection of human dermal fibroblasts from oxidative stress. *J Dermatol Sci*. 2008;49:133–142.
133. Chae YB, Lee JS, Park HJ, et al. Advanced adipose-derived stem cell protein extracts with antioxidant activity modulates matrix metalloproteinases in human dermal fibroblasts. *Environ Toxicol Pharmacol*. 2012;34:263–271.
134. Svineng G, Ravuri C, Rikardsen O, et al. The role of reactive oxygen species in integrin and matrix metalloproteinase expression and function. *Connect Tissue Res*. 2008;49:197–202.
135. Du Y, Roh DS, Funderburgh ML, et al. Adipose-derived stem cells differentiate to keratocytes in vitro. *Mol Vis*. 2010;16:2680–2689.
136. Hu R, Xu W, Han D. Fibroblast-like cells differentiated from adipose-derived mesenchymal stem cells for vocal fold wound healing. *PLoS One*. 2014; 9:e92676.
137. Hasegawa T, Sakamoto A, Wada A, et al. Keratinocyte progenitor cells reside in human subcutaneous adipose tissue. *PLoS One*. 2015;10:e0118402.
138. Bourlier V, Zakaroff-Girard A, Decaunes P, et al. TGFbeta family members are key mediators in the induction of myofibroblast phenotype of human adipose tissue progenitor cells by macrophages. *PLoS One*. 2012;7:e31274.
139. Diaz-Flores L, Gutiérrez R, García MP, et al. $CD34^+$ stromal cells/fibroblasts/fibrocytes/telocytes as a tissue reserve and a principal source of mesenchymal cells. Location, morphology, function and role in pathology. *Histol Histopathol*. 2014;29:831–870.
140. Sivan U, Jayakumar K, Krishnan LK. Matrix-directed differentiation of human adipose-derived mesenchymal stem cells to dermal-like fibroblasts that produce extracellular matrix. *J Tissue Eng Regen Med*. 2016;10:E546–E558.
141. Plikus MV, Guerrero-Juarez CF, Ito M, et al. Regeneration of fat cells from myofibroblasts during wound healing. *Science*. 2017;355:748–752.
142. Carceller MC, Guillén MI, Ferrándiz ML, et al. Paracrine in vivo inhibitory effects of adipose tissue–derived mesenchymal stromal cells in the early stages of the acute inflammatory response. *Cytotherapy*. 2015;17:1230–1239.
143. Manning CN, Martel C, Sakiyama-Elbert SE, et al. Adipose-derived mesenchymal stromal cells modulate tendon fibroblast responses to macrophage-induced inflammation in vitro. *Stem Cell Res Ther*. 2015;6:74.
144. Shang Q, Bai Y, Wang G, et al. Delivery of adipose-derived stem cells attenuates adipose tissue inflammation and insulin resistance in obese mice through remodeling macrophage phenotypes. *Stem Cells Dev*. 2015;24:2052–2064.
145. Yin X, Pang C, Bai L, et al. Adipose-derived stem cells promote the polarization from M1 macrophages to M2 macrophages [in Chinese]. *Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi*. 2016;32:332–338.
146. Castagnoli C, Trombotto C, Ondei S, et al. Characterization of T-cell subsets infiltrating post-burn hypertrophic scar tissues. *Burns*. 1997;23:565–572.
147. Foley TT, Ehrlich HP. Through gap junction communications, co-cultured mast cells and fibroblasts generate fibroblast activities allied with hypertrophic scarring. *Plast Reconstr Surg*. 2013;131:1036–1044.
148. Cui L, Yin S, Liu W, et al. Expanded adipose-derived stem cells suppress mixed lymphocyte reaction by secretion of prostaglandin E_2. *Tissue Eng*. 2007;13: 1185–1195.
149. Gimble JM, Bunnell BA, Frazier T, et al. Adipose-derived stromal/stem cells: a primer. *Organogenesis*. 2013;9:3–10.
150. Kim JH, Lee YT, Hong JM, et al. Suppression of in vitro murine T cell proliferation by human adipose tissue–derived mesenchymal stem cells is dependent mainly on cyclooxygenase-2 expression. *Anat Cell Biol*. 2013;46:262–271.
151. Dave M, Hayashi Y, Gajdos GB, et al. Stem cells for murine interstitial cells of Cajal suppress cellular immunity and colitis via prostaglandin E_2 secretion. *Gastroenterology*. 2015;148:978–990.
152. Sheng J, Chen W, Zhu HJ. The immune suppressive function of transforming growth factor-β (TGF-β) in human diseases. *Growth Factors*. 2015;33:92–101.
153. Li R, Li H, Sun Q, et al. Indoleamine 2,3-dioxygenase regulates T cell activity through Vav1/Rac pathway. *Mol Immunol*. 2016;81:102–107.
154. Liu YL, Liu WH, Sun J, et al. Mesenchymal stem cell–mediated suppression of hypertrophic scarring is p53 dependent in a rabbit ear model. *Stem Cell Res Ther*. 2014;5:136.
155. De Felice B, Garbi C, Santoriello M, et al. Differential apoptosis markers in human keloids and hypertrophic scars fibroblasts. *Mol Cell Biochem*. 2009; 327:191–201.
156. Li Y, Zhang W, Gao J, et al. Adipose tissue–derived stem cells suppress hypertrophic scar fibrosis via the p38/MAPK signaling pathway. *Stem Cell Res Ther*. 2016;7:102.
157. Zhang GY, Li X, Yi CG, et al. Angiotensin II activates connective tissue growth factor and induces extracellular matrix changes involving Smad/activation and p38 mitogen-activated protein kinase signalling pathways in human dermal fibroblasts. *Exp Dermatol*. 2009;18:947–953.
158. Du QC, Zhang DZ, Chen XJ, et al. The effect of p38MAPK on cyclic stretch in human facial hypertrophic scar fibroblast differentiation. *PLoS One*. 2013; 8:e75635.
159. Chen JY, Zhang L, Zhang H, et al. Triggering of p38 MAPK and JNK signaling is important for oleanolic acid-induced apoptosis via the mitochondrial death pathway in hypertrophic scar fibroblasts. *Phytother Res*. 2014;28:1468–1478.