The molecular basis of keloid and hypertrophic scar formation

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Excess scar formation secondary to traumatic or surgical injuries can have devastating consequences, ranging from body disfigurement to organ dysfunction. Hypertrophic scars and keloids are skin fibrotic conditions that can be caused by minor insults to skin, such as acne or ear piercing, or by severe injuries such as burns. Differences between keloids, hypertrophic scars and normal scars include distinct scar appearance, histologic morphology and cellular function in response to growth factors. Recent advances in our understanding of the wound healing process reveal possible causes for hypertrophic scars and keloids. This information might assist in the development of efficacious treatment for hypertrophic scar and keloid formation.

KELOIDS and hypertrophic scars are skin abnormalities that are unique to humans and are characterized by excessive deposition of collagen in the dermis and subcutaneous tissues secondary to traumatic or surgical injuries. Contrary to the asymptomatic fine-line scar characteristics of normal wound repair, the exuberant scarring of keloid and hypertrophic scars results typically in disfigurement, contractures, pruritis and pain.

Clinically, keloids are defined as scars growing beyond the confines of original wounds, which rarely regress over time. It has been estimated that ~15–20% of Blacks, Hispanics and Orientals suffer from keloids and there appears to be a genetic predisposition to keloid formation. Genetic approaches, such as linkage analysis, are being taken to identify the chromosomal location of the predisposing gene for the ultimate purpose of cloning and characterizing the genetic lesion. Hypertrophic scars, on the other hand, are raised scars that remain within the boundaries of the wound and frequently regress spontaneously (Fig. 1). Histologically, collagen bundles in the dermis of normal skin, or normal scar tissues, appear relaxed and are arranged in a random array (Fig. 2a). Keloids and hypertrophic scars have collagen bundles that appear stretched and aligned in the same plane as the epidermis (Fig. 2b,c). These collagen bundles are thicker and more abundant in keloid scars and form acellular node-like structures in the deep dermal portion of the keloid lesion (Fig. 2c). The center of keloid lesions contain relatively few cells compared with hypertrophic scars. Apoptosis may be involved in the clearance of certain cell populations in keloid lesions1. In contrast, islands composed of aggregates of fibroblasts, small vessels and collagen fibers are seen throughout the dermis of hypertrophic scars2. Minor insults to the skin, such as acne eruptions or ear piercing, can produce large keloid lesions. These lesions are often refractory to medical and surgical treatment. Hypertrophic scars result from injury to the deep dermis, particularly traumatic wounds and wounds that manifest a prolonged phase of inflammation and fibrosis. Their size is commensurate with the extent of injury and does not involve undamaged peripheral skin. Mechanical stress, age, body location and circulating growth factors and hormones affect the development of these scars. Often, hyper-
trophic scars can be improved with appropriate surgical scar revision. In many individuals, however, neither surgery nor pharmacological measures prove to be helpful for keloids or hypertrophic scars, underlining the vital need to understand and control these disfiguring maladaptive processes.

In this review, we do not intend to cover a wide spectrum of hypotheses that attempt to explain the genesis of keloids and hypertrophic scars. Rather, we will focus on the most recent research and relate key findings to the etiology of keloid and hypertrophic scar formation.

Cellular and molecular mechanisms of normal injury repair

Tissue repair is accomplished through concerted events involving various cell types, extracellular matrix (ECM) components, cytokines and other soluble mediators. Skin repair begins with the formation of a fibrin-rich blood clot, which provides a provisional matrix for reparative events to follow. This process ends with newly synthesized scar tissue composed mainly of collagen, which restores the functional integrity of the skin. Several vital sequential stages have been identified in the repair process, namely inflammation, fibroplasia, formation of granulation tissue and scar maturation. The dynamic interactions and feedback control mechanisms among participating components in these different stages govern the direction of the repair. Therefore, an aberration in the process can result in poorly healing chronic wounds at one extreme and excessively healing hypertrophic scars and keloids at the other. It could be postulated further that even more extreme fibroproliferative disease processes, such as Peyronie’s and Dupuytren’s disease, plantar fascitis, fibromatosis, desmoid tumor and fibrosarcoma, might share similar cellular and molecular mechanisms that have become even more unrestrained.

The use of in vitro systems has aided the identification and characterization of many of the components that interact during wound healing. For instance, there are many compelling in vitro examples of the control of cellular gene expression through the adhesive interaction of connective tissue cells with their surrounding ECM. Many of these interactions are mediated through cell adhesion receptors called integrins. Frequently, their expression is regulated by cytokines and growth factors, such as transforming growth factor β (TGF-β), released from adjacent cells or the surrounding ECM through limited proteolysis. Indeed, proteolytic degradation of ECM is an essential feature of tissue repair and remodeling processes. The serine proteases, including plasminogen activator (PA)-plasmin and the matrix metalloproteinases (MMPs), are the two major groups of ECM-degrading enzymes that interact and form a lytic cascade for ECM remodeling. The major function of PA is to control the activation of plasminogen into plasmin. Plasmin is not only the primary effective enzyme in fibrinolysis, but it also participates in the degradation of ECM and activates procollagenase into collagenase (a member of the MMP family). Thus, the initiation of the protease cascade by PA leads to a notable amplification of proteolytic activity. The complexity of this regulatory system is increased by the fact that plasmin can release active TGF-β from its latency-associated protein.
In turn, TGF-β regulates plasminogen activator inhibitor 1 (PAI-1), MMPs, tissue inhibitor of metalloproteinases 1 (TIMP-1) and genes encoding ECM components and their integrin receptors. A simplified synopsis of the control-feedback loops consists of the ECM-degrading enzymes that interact and form a lytic cascade for ECM remodeling. Plasmin is not only the primary effective enzyme in fibrinolysis, but also activates procollagenase into collagenase which degrades collagen. Plasmin activation is inhibited by PAI while collagenase activity is inhibited by TIMP. In turn, TGF-β regulates plasminogen activator inhibitor 1 (PAI-1), MMPs, tissue inhibitor of metalloproteinases 1 (TIMP-1) and genes encoding ECM components and their integrin receptors. A simplified synopsis of the control-feedback loops consists of the ECM-degrading enzymes that interact and form a lytic cascade for ECM remodeling. Plasmin is not only the primary effective enzyme in fibrinolysis, but also activates procollagenase into collagenase which degrades collagen. Plasmin activation is inhibited by PAI while collagenase activity is inhibited by TIMP. Heterotrophic scar and keloid fibroblasts exhibit differences in growth factor-mediated ECM remodeling and synthesis. Dermal fibroblasts play a major role in scar formation and have been used in vitro for a variety of wound repair studies to uncover the mechanism of fibrosis. Often, as in vitro these fibroblasts exhibit characteristics typical of their in vivo phenotypes. For instance, keloid fibroblasts continue to produce high levels of collagen, fibronectin, elastin and proteoglycan in vitro and show aberrant responses, compared with normal fibroblasts, to metabolic modulators such as glucocorticoids, hydrocortisone, growth factors and phorbol esters. The altered response of keloid fibroblasts to these metabolic modulators is thought to contribute to the pathogenesis of keloid formation. Fibroblasts from hypertrophic scars also display a moderate elevation in collagen production in vitro, however, their response to the metabolic modulators are similar to normal fibroblasts.

Tissue repair is governed by a state of finely controlled equilibrium between matrix synthesis and degradation. Using an in vitro fibrin-plasmin model, we discovered a striking increase of PAI-1 with a concomitant decrease of urokinase plasminogen activator (uPA) in keloid fibroblasts. As a result, keloid fibroblasts exhibit a decreased capacity for fibrinolysis and, therefore, fibrin clot degradation. The increase of PAI-1 expression is found at both the mRNA and protein levels and is unique to keloid fibroblasts. Although still poorly understood, the elevated PAI-1 levels exhibited by keloid fibroblasts may also have significant consequences for the repair steps that follow fibrin clot removal. For instance, a reduction in plasmin generation caused by increased PAI-1 activity might subsequently result in a reduction in plasmin-mediated collagenase activation and plasmin-dependent collagen degradation by collagenases. Degradation and remodeling of newly synthesized collagen in the wound are important steps in both removing excess collagen produced by fibroblasts and scar maturation. It is conceivable, therefore, that the excessive collagen present in keloid lesions might result from not only an increased collagen production by keloid fibroblasts, but also a decrease in collagen degradation and removal during scar remodeling.

Scar contracture is another important difference between hypertrophic scars and keloids. Scar contracture results from excessive contraction of ECM by fibroblasts in the wound. It produces severe clinical consequences ranging from joint contracture with range-of-motion loss to body disfigurement. Scar contracture is often observed in hypertrophic scars but not in keloids. A similar process might be involved in other medical disease processes such as Peyronie’s and Dupuytren’s disease, plantar fascia and fibromatoses. An in vitro analysis of ECM contraction by fibroblasts isolated from different scars shows that hypertrophic scar fibroblasts exhibit stronger fibrin clot contraction than normal or keloid fibroblasts. This heightened contractility can be attributed to TGF-β through autocrine control, because these cells secrete increased levels of TGF-β and a neutralizing antibody to TGF-β can attenuate the contraction. Although the mechanism underlying TGF-β-mediated matrix contraction by fibroblasts is...
unknown at present, it has been demonstrated that TGF-β can induce the expression of smooth muscle (α-SM) actin, a major component of the cytoskeleton that participates in cell adhesion and locomotion. Indeed, hypertrophic scars contain abundant myofibroblasts rich in α-SM actin (myofibroblasts originate from migrating fibroblasts during the formation of granulation tissue). The mechanism by which wound fibroblasts or myofibroblasts exert force on the surrounding ECM is only beginning to be understood. Studies using in vitro collagen matrix gels demonstrate that skin fibroblasts can reorganize collagen matrix to cause matrix contraction. In turn, the subsequent tension that develops in the matrix causes fibroblast proliferation and biosynthetic activity to be upregulated. The subsequent development of tension in the matrix is thought to be mediated by TGF-β. TGF-β serves multiple functions in tissue/organ repair by increasing cellular production of ECM components, such as fibronectin and collagen, and also increases the cellular expression of integrins (not shown). Furthermore, the synthesis of inhibitors of degradation enzymes such as plasminogen activator inhibitor (PAI) and tissue inhibitor of matrix metalloproteinases (TIMP) are also increased by TGF-β, while the expression of collagenase and plasminogen activator (PA) are decreased. This upregulation of inhibitor synthesis and downregulation of protease synthesis further augments the accumulation of ECM proteins induced by TGF-β, and is the basis for fibrotic tissue formation due to excessive action of TGF-β. Possible means of therapeutic intervention are high-litigated. Antagonists of TGF-β and its receptor would shift the ECM equilibrium towards degradation, as would upregulators of PA production and PA analogs. Inhibitors of collagen and ECM synthesis would prevent excessive ECM deposition. Cell-cycle inhibitors would prevent the proliferation of fibroblasts.

Therefore, experimental evidence suggests that fibroblasts from hypertrophic scars might represent a hyperproliferative phenotype resulting from multiple stimulatory effects present in the wound environment. This phenotype can be reverted once the stimulation, such as the over-abundance of growth factors or tension of the skin, is lifted. Keloid fibroblasts, on the other hand, represent a unique phenotype that is genetically predisposed to changes in ECM production and PAI-1 expression. This altered phenotype is switched on irreversibly after wounding by factors such as TGF-β.

**TGF-β signaling mechanisms**

TGF-β is secreted by a variety of cells and serves multiple functions in tissue/organ repair by increasing cellular production of ECM components, such as fibronectin and collagen, and cell-cell expression of matrix receptor integrin. Furthermore, the synthesis of PAI-1 and TIMP is also increased by TGF-β, while the expression of collagenase and PA is decreased. This upregulation of inhibitor synthesis and downregulation of protease synthesis further augments the accumulation of ECM proteins induced by TGF-β, and is the basis for fibrotic tissue formation caused by the excessive action of TGF-β (Fig. 4).

The mechanism of receptor signaling holds the key to TGF-β regulation of cellular responses and is one of the most intensively studied areas of TGF-β research. TGF-β and its family members, activin and bone morphogenetic protein (BMP), signal through heteromeric transmembrane serine/threonine kinases known as type I and type II receptors. The type II receptor is activated constitutively. It recruits the type I receptor by means of bound TGF-β and subsequently phosphorylates the type I receptor. Many type I-like receptor proteins have been identified and the biological response to TGF-β in a given cell type appears to be defined by the particular type I receptor engaged in the complex. Receptor activation leads to the phosphorylation of receptor-associated TAK1-binding proteins (TAK1s), which activate the TAK1 kinase cascade, or SMADs, a set of evolutionary conserved proteins that translocate to the nucleus to activate transcription. Keloid fibroblasts respond to TGF-β by further increasing their already augmented rate of collagen synthesis, a phenomenon not detected in fibroblasts of normal scar or hypertrophic scar origin. Keloid fibroblasts, nevertheless, secrete levels of TGF-β comparable to that of normal fibroblasts, thus, the altered response or unique sensitivity of keloid fibroblasts to TGF-β might reflect a change that occurs at the receptor level or postreceptor signaling.

**Conventional treatments for hypertrophic scars and keloids based on information from laboratory findings**

In the past, several drugs have been investigated for the purpose of inhibiting collagen synthesis and accelerating the removal of excessive collagen deposited in hypertrophic and keloid scars. Historically, these drugs have included collagen crosslinking inhibitors, β-amino acid inhibitors of fibrinolysis and PA production, and inhibitors of cell-cycle progression. However, the most promising treatment has been inhibitors of the TGF-β signaling pathway. Antagonists of TGF-β and its receptor would shift the ECM equilibrium towards degradation, as would upregulators of PA production and PA analogs. Inhibitors of collagen and ECM synthesis would prevent excessive ECM deposition. Cell-cycle inhibitors would prevent the proliferation of fibroblasts.
tested clinically and demonstrated an average of 30.4% reduction in fibrotic agents. In particular, recombinant interferon suppress the synthesis of collagen, have been used as anti-
b and well-defined and controllable parameters of cell type and number, logical scarring while at the same time avoiding untoward sequelae. precise intervention will be required for beneficial treatment of patho-
cacy, timing and optimal dosage of these potential agents for clinical liferative disorders, further studies are still required to establish effi-
sive action in promoting tissue fibrosis. Approaches taken to antago-
tiation, but also because of direct experimental evidence of its exces-
punctual role in regulating both cell proliferation and differen-
and interferons has been shown promise30.

Currently, clinicians and basic scientists are actively pursuing arti-
ficial and natural antagonists to factors that regulate the phenotypes of connective tissue cells during repair. The purpose is to attenuate or regu-
ate the excessive cell proliferation and synthesis and contraction of ECM during repair by scar fibroblasts. Among these factors, TGF-β has received the most attention not only based on its proven multifunctional role in regulating both cell proliferation and differen-
tation, but also because of direct experimental evidence of its exces-
sive action in promoting tissue fibrosis. Approaches taken to antago-
ize TGF-β-stimulated fibrous include the use of neutralizing anti-TGF-β antibodies11,24, the naturally occurring TGF-β-binding proteoglycan decorin25 and mannos-6-phosphate, an antagonist of TGF-β activation26. Other growth factors, such as PDGF27 and con-
nective tissue growth factor (CTGF)28, have also been implicated in fibrosis and are targets for the blockade of fibrosis. Cytokines such as interleukin 1, tumor necrosis factor α and interferons γ and α, which suppress the synthesis of collagen, have been used as anti-
fibrotic agents. In particular, recombinant interferon γ has been tested clinically and demonstrated an average of 30.4% reduction in keloid scar thickness29. Calcium antagonists such as verapamil, which affect cytoskeleton reorganization and induce procollagenase synthesis by fibroblastic cells, have been tried in limited clinical trials through intralesional injection into hypertrophic scars, and appear to show promise30.

Despite the recent advancement in therapeutic designs for fibrop-
liferative disorders, further studies are still required to establish effi-
cacy, timing and optimal dosage of these potential agents for clinical application. In addition, most of the target agents are produced by cells during skin wound repair, and their proper temporal and spatial expression during repair is required for normal healing. Therefore, precise intervention will be required for beneficial treatment of patho-
logical scarring while at the same time avoiding untoward sequelae.

Future directions

The complex nature of the repair process and the lack of proper in vitro and in vivo animal models for scar formation have hindered progress in revealing the mechanisms of pathologic scar formation. Although in vitro studies have the advantages of investigating hypoth-
eses under defined systems, the results do not always reflect the in vivo situation. Therefore, the development of appropriate culture sys-
tems that reflect the in vivo condition more closely is required. In this regard, three-dimensional fibrin model system features key characteristics of fibrosis, i.e. fi-
brin matrix reorganization (matrix gel contraction), cell proliferation, fibrin degradation and collagen synthesis/deposition. Therefore, the system is well suited for some creative designs in studies of mecha-

Glossary

Desmoid tumor – A nodule or relatively large mass of unusually firm scar-like connective tissue resulting from active proliferation of fi-
broblasts.

Dyspyrogen’s disease – A condition marked by fibromatoses of the connective tissue of the foot. Pathologically, it is characterized by the presence of masses of proliferating fibroblasts.

Fibromatoses – Abnormal hyperplasia of the fibrous tissue.

Fibroplasia – Production of fibrous tissue.

Granulation tissue – Newly formed connective tissue that fills the defect of a wound.

Hypertrophic scar – Raised fibrous connective tissue in the dermis and adjacent subcutaneous tissue after traumatic or burn wound healing.

Integrin – Member of the large family of transmembrane proteins involved in the adhesion of cells to the extracellular matrix.

Keloid – A nodular, frequently lobulated, firm, movable, non-
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Transgenic animals also provide a new approach to the investi-
gation of gene function in vivo. Definitive proof of the involvement of plasmin in wound repair is provided by plasminogen-deficient mice32. These mice exhibit severely impaired healing of skin wounds, abnor-
mal keratinocyte migration and protrusion of excessive granulation tissue in the middle of the wound, resembling a raised scar. Although this animal model is not an ideal substitution for human studies, it might be a first step towards investigating the role of the plasmino-
gen-plasminogen-activator protease system in tissue repair.

Mechanisms of fetal wound repair have attracted much attention in recent years, owing to the observation that fetal wounds heal without

and matrix type and concentration. The culture begins with intimate, uniform cellular contact with the ECM and progresses to remodeling. Experimental reproducibility and a microcell format provide feasibil-
ity for pharmacological studies. Furthermore, the three-dimensional fibron gel model system features key characteristics of fibrosis, i.e. fi-
brin matrix reorganization (matrix gel contraction), cell proliferation, fibrin degradation and collagen synthesis/deposition. Therefore, the system is well suited for some creative designs in studies of mecha-

system under both normal and abnormal healing processes. Transgenic animals also provide a new approach to the investi-
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References


The outstanding questions

- What is the genetic basis of keloid formation?
- What are the key control and feedback points for molecular and cellular events in wound repair?
- What is the mechanism underlying altered responsiveness of keloid fibroblasts to growth factors, such as TGF-beta, at the levels of growth factor receptors and post-receptor signaling events?
- What are the best experimental models for hypertrophic scar and keloid research?
- What is the turning point from fetal scarless healing to scarring, and how can we apply the information gained by studying fetal wound repair to the treatment of hypertrophic scar and keloid formation?