Cellular Signaling in Tissue Regeneration

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Abstract

With recent progress in stem cell-based research, there has been tremendous interest in stem cell-based tissue regeneration. Stem cells can be differentiated into specialized cells/tissues by growth factors and cytokines. These small molecules are thought to play an important role in both wound healing and tissue regeneration. However, their biological activity and signal transduction during tissue regeneration are poorly understood. With recent advances in signal transduction by growth factors, the receptor kinases and G protein-coupled receptors, an understanding in the underlying mechanism of how these factors regulate tissue regeneration beginning to take place. In this review, the potential underlying mechanisms of growth factor signaling in normal tissue regeneration and chronic wound healing is discussed. Thus, it is an aim to provide a basis for designing more specific therapies for tissue regeneration in the near future.

Key Words: Growth factors, cytokines, chemokines, MCP-1, CCR2, receptors, keloids, hypertrophic scars, fibroproliferative disease, collagen, signal transduction, wound repair, injury, regeneration, stem cells, tissue engineering

INTRODUCTION

The successful application of nuclear transfer techniques to a range of mammalian species has brought the possibility of stem cell-based human cell regeneration therapies. Pluripotent stem cells that carry the nuclear genome of the patient can be produced and then be forced to differentiate into replacement cells, such as cardiomypocytes that can replace damaged heart tissue or insulin-producing beta cells for patients with diabetes.\(^1,3\) Although this approach would eliminate the critical problem of immune incompatibility, there is also the task of reconstituting the cells into more complex tissues and organs in vitro.

Committed stem and progenitor cells have been isolated from various adult tissues, including hematopoietic stem cells, neural stem cells, hair follicle stem cells, mesenchymal stem cells and endothelial progenitor cells. These adult stem cells have several advantages over embryonic stem cells in their practical therapeutic application for tissue regeneration.\(^1,4-8\)

For a promising gene therapy, the application of adult stem and progenitor cells in terms of modifying stem cell potency, altering organ properties, accelerating regeneration and forming an expressional organization, the basic mechanisms of tissue regeneration in the human body need to be understood.

Tissue regeneration is a complex cellular process, which includes the processes of inflammation, angiogenesis, extracellular matrix synthesis, reepithelialization and collagen deposition.\(^3,9,10\) The complexity and clinical variability of wound healing has limited pharmacologic approaches to accelerate wound repair. Until recently, no specific pharmacologic agents that could reproducibly accelerate wound healing had been identified.\(^11\) Growth factors discovered in the processes of wound repair have opened the door to new therapies that can manipulate wound repair.\(^12,13\)

Growth factors such as the platelet derived growth factor (PDGF), the epidermal growth factor (EGF), and the fibroblast growth factor (FGF) are the ligands for receptor tyrosine kinases. The receptor tyrosine kinases exhibit a similar molecular structure and are activated by a common mechanism. The mechanism for ligand-induced activation of the receptor tyrosine kinases is well established.\(^14-17\) Binding of a ligand to the extracellular domain induces receptor dimerization, which activates the catalytic domain of protein tyrosine kinase and leads to tyrosine autophosphorylation.\(^14,18\) Phosphorylation of tyrosines within the
catalytic domain is essential for maintaining the tyrosine kinase in an active state. The residues of the phosphorylation, which are located in noncatalytic regions, generates docking sites for the SH2 (Src homology 2) and PTB (phosphotyrosine binding) domains of the signaling molecules. A variety of signaling proteins are directly recruited by activated receptors, while other signaling molecules are activated by tyrosine phosphorylation. In many cases, the growth factor receptor ligands are soluble proteins. Such soluble growth factors activate their specific cell surface receptors by multivalent interactions and exert their biological effects by endocrine, paracrine, or autocrine mechanisms.

TGF-β signaling requires a heteromeric assembly of its two serine-threonine kinase receptors, designated RI and RII, respectively. A recent model illustrating both the physical and functional interactions between the two receptors proposes that upon ligand binding, the constitutively active RII recruits RI and transphosphorylates the RI, which subsequently initiates downstream cytoplasmic events. The discovery of SMAD (mammalian homolog of Drosophila Mad gene) proteins has allowed the delineation of a mechanism by which TGF-β and related growth factors convey their signals from the membrane receptors into the nucleus. The SMADs are directly phosphorylated and activated by the receptors where they then form heteromeric SMAD-SMAD complexes that move into the nucleus. From the nucleus, they orchestrate many transcriptional responses. Different modes of the SMAD interactions are regulated by phosphorylation. The SMAD domains that mediate these SMAD interactions i.e. binding to DNA or transcriptional activation have been defined. The recent discovery of antagonistic SMADs and a regulatory crosstalk with the Ras/MAP-kinase pathways have added directly to the rapidly expanding understanding of this major regulatory network. Defects in the expression of either receptor would contribute to the loss of exogenous and endogenous TGF-β response. Interestingly, in some fibrotic diseases, the expression levels of the SMAD molecules are quite different, when compared to normal human skin (Kim et al., unpublished data). Further investigation is necessary to elucidate whether the SMAD molecules play a role in both normal wound repair and fibrotic diseases.

Chemokine signaling

Monocyte Chemoattractant Protein-1 (MCP-1): Chemokines or chemoattractant cytokines represent an expanding family of structurally related low molecular weight proteins that are recognized as being responsible for both leukocyte trafficking and activation. Soon after the discovery of this class of cytokines, the monocyte chemoattractant protein-1 (MCP-1) was found to be expressed strongly in human atherosclerotic lesions. This protein was postulated to be central in monocyte recruitment into the arterial wall and the developing lesions. In this review, our present knowledge regarding MCP-1 and its receptor CCR2, and their role in both atherogenesis and wound healing will be discussed.

Although less well established, other chemokines such as RANTES, MIP-1alpha and MIP-1beta have also been implicated in atherosclerotic lesion formation as have a number of other more recently discovered chemokines like MCP-4, ELC and PARC. The role of these chemokines in the progression of atherosclerosis will be discussed as well as the emerging role of IL-8; mostly known for its effects on neutrophils. Particular attention will be given not only to the involvement of chemokines in the inflammatory recruitment of monocytes/macrophages, but also to their role in related local immune responses and vascular remodelling that occur during the formation of unstable atherosclerotic plaques.

Endothelial cell proliferation and migration may play a central role in wound healing, angiogenesis and atherosclerosis. Direct evidence for the function of MCP-1 and its receptor, CC chemokine receptor 2 (CCR2), in the pathogenesis of atherosclerosis has been provided from studies using mice lacking the CCR2 receptor, which were crossed with ApoE knockout mice. The CCR2 (-/-) mice showed significant defects in leukocyte adhesion, monocyte/macrophage recruitment and a reduction in INF-g production when exposed to both antigenic and nonantigenic stimuli. Upon a femoral artery denudation injury, the CCR2 (-/-) mice exhibited similar effects (Kim et al., manuscript in preparation). When these mice were crossed with the ApoE (-/-) and fed a western type diet, they displayed a marked decrease in lesion formation. Not only was there a decrease in lesion size, but also a decrease in the number of macrophages in these lesions. These results support the hypothesis that MCP-1 is a major chemokine.
involved in monocyte recruitment during atherosclerosis. Moreover, the process of monocyte recruitment is a major determinant of lesion size and complexity.

Although CXC chemokines can act on the endothelial cells by influencing proliferation, the involvement of CC chemokines and endothelial expression of the chemokine receptors in wound healing remains to be elucidated. Reverse transcription-polymerase chain reaction, RNase protection, Western blot, and flow cytometric analysis showed that human umbilical vein endothelial cells express the mRNA and surface protein of the monocyte chemotactic protein-1 (MCP-1) receptor, CCR2, which was upregulated by inflammatory cytokines. MCP-1 induced endothelial cell migration in a transwell assay, which was inhibited by the 9–76 MCP-1 receptor antagonist. Increased secretion of MCP-1 or interleukin-8, but not RANTES, after an endothelial injury suggested a functional role of CCR2 in wound repair as measured by ELISA. After mechanical injury to the endothelial monolayers, which spontaneously closed within 24 hours, wound repair was delayed by the 9–76 antagonist and by a blocking monoclonal antibody to MCP-1, but not to interleukin-8. However, wound repair was improved by exogenous MCP-1. This was confirmed by quantifying the cell migration into the wound area, whereas both proliferation and viability were unaltered by MCP-1 or its analogue. Notably, the immunohistochemistry of the inflamed tissue revealed CCR2 staining on arterial, venous, and venular endothelium that was affected by cellular infiltration. Thus the endothelial cells express the functional CCR2, which may have important implications for both endothelial wound repair and inflammatory reactions.

**Growth factor signaling**

**Platelet-Derived Growth Factor (PDGF):** In both animal experiments and human clinical trials, PDGF has been shown to be a potent stimulator for wound healing. PDGF enhances angiogenesis and epithelialization in excisional wound models and increases the breaking strength of incisional wounds in both normal and impaired healing models. PDGF also accelerates the deposition of the provisional wound matrix containing, in particular, glycosaminoglycans and fibronectin. These results suggest that PDGF plays an important role in wound healing. However, its underlying mechanism is still unclear.

The PDGF receptor signal transduction mechanisms have been extensively studied. PDGF induces tyrosine phosphorylation of the PDGF receptor and numerous other intracellular proteins. The PDGF receptor mediates fibroblast chemotaxis, proliferation, and induction of both the extracellular matrix and metalloproteinases, which are required for wound remodeling. Until recently, it was not known which molecules were involved in PDGF-mediated wound healing. However, it is now known that PDGF activates the small GTPase Rac in the fibroblasts. Gelsolin, as described earlier, is a downstream effector of Rac. Therefore, it is most likely that Rac may be one of the key molecular switches responsible for the onset of PDGF-mediated fibroblast migration into a wound. In order to understand fully the mechanisms of PDGF-mediated wound repair, further studies are needed to identify the PDGF receptor-secondary signaling molecules. Furthermore, it is necessary to determine the specificity of the tyrosine kinases in accelerating wound healing. PDGF may be an important future clinical tool, particularly for stimulating soft tissue repair in patients with an impaired capacity for wound healing. However, in order to optimize the therapeutic parameters, e.g., dose, methods of administration, and choice of PDGF isoform, much more work is needed. Moreover, for different types of wounds, critical comparisons with other growth factors should be performed in order to select the best factor, or combinations of factors.

**Epidermal Growth Factor (EGF):** In the numerous therapeutic studies, EGF significantly enhanced wound healing. EGF has been shown to stimulate epidermal repair in animal excisional and thermal injury models. It may also stimulate dermal repair and appears to be effective in accelerating repair of chronic ulcers. EGF also accelerated the closure of small wounds in the confluent guinea-pig airway epithelial monolayers.

To gain insight into the participation of the EGF receptor and its ligands in wound repair, the expression of the EGF receptor was examined in normal neonatal and adult skin by immunohistochemistry and in situ hybridization. Injections of EGF into neonatal mice showed that EGF could affect epithelial structures by modulating the normal developmental process. The EGF receptor has been localized through-
out all nucleated layers of the neonatal epidermis. However, in the adult dermis, the EGF receptor is spatially positioned only on the basal layer keratinocytes, which are cells of prime importance for resurfacing human partial-thickness wounds. The presence or absence of the EGF receptor in the epidermis and within the epidermal appendages in specific keratinocyte populations, implies both a regulatory and biological significance for this cytokine signaling pathway. Since the skin appendages are situated deep within the dermis and subcutaneous tissues, this population of keratinocytes is better protected from the environment and, therefore, serves as a ready source of replacement cells for the overlying epidermis lost during wound formation. During wound healing, EGF receptor-mediated events appear to play a critical role in reforming the epidermal permeability barrier, keratinocyte differentiation, keratinocyte proliferation, and keratinocyte migration and adhesion. However, the underlying mechanisms by which EGF regulates repair remain undefined.

To further investigate the functional role of EGF in wound healing, the EGF receptor-signal-transduction mechanisms have been extensively studied. In the EGF receptor-signal-transduction pathway, we are now able to trace the molecules relaying signals from the receptor to the nucleus, such as Grb2. In addition, much attention has been paid to the protein tyrosine phosphatases (PTPases). Identification of a large family of PTPases has lead to the study of tyrosine kinase interactions such as the EGF receptor with PTPases. PTPases have been localized in normal human skin, but have not been examined in terms of wound healing. Since stimulatory signals through the tyrosine kinase receptors such as the EGF receptor must be attenuated with regulatory molecules such as the PTPases, studying the potential role of PTPases in wound healing will be of great value.

The remarkable ability of the fetus to heal early gestation skin wounds without scarring is poorly understood. Taking advantage of recent advances in signal transduction studies, the tyrosine phosphorylation patterns of fetal rat fibroblasts, representing the scarless cutaneous repair phenotype, and adult rat fibroblasts, representing the scar-forming phenotype, were examined to determine whether there were inherent differences in cellular signaling. Specifically, the main aim was to correlate the phosphorylation patterns with the expression levels of the signaling molecules, which transmit information from the plasma membrane receptor to the nucleus. By using three different cell lines of the explanted fibroblasts from fetal rat skin, obtained 13 days after gestation (n=24), and 1-month-old postnatal adult rat skin (n=3), immunoblotting was performed to compare tyrosine phosphorylation patterns. The results revealed five major protein bands of interest in fetal rat fibroblasts, but not in the adult rat fibroblasts. These phosphorylated protein bands are of interest because of their possible role in wound repair and may have the potential to regulate cellular responses to the extracellular matrix and their secondary signaling molecules. It was hypothesized that these bands represented the receptor tyrosine kinases, the epidermal growth factor receptor, the discoidin domain receptor, and their downstream adaptor protein. She that binds the receptor tyrosine kinases to transduce signals intracellularly. Furthermore, the elevated expression of the platelet-derived growth factor receptor-beta in adults compared with fetal fibroblasts was demonstrated. This suggested that there was a decreased expression of certain growth factors, which may also be important for the scarless phenomenon to occur.

Until recently, it was not clear how the extracellular signals might affect the cell motility involved in wound healing. However, it is now known that the EGF ligand binding to the receptor activates the signaling molecule, MEK, and that MEK selectively interacts with the small guanosine triphosphatase (GTPase) Rac/Cdc42. When the Rac is activated in the fibroblasts in response to EGF, it transmits signals leading to actin-based membrane ruffling, which mediates lamellipodial extension and the assembly of focal adhesion complexes as part of the crawling response of tissue culture fibroblasts and epithelial cells. Interestingly, recent studies have shown that gelsolin, crucial for actin filament organization, is a downstream effector of Rac for fibroblast motility. Compared to the wild type dermal fibroblasts, gelsolin-null dermal fibroblasts showed a significantly reduced ruffling response to EGF. Stable expression of gelsolin in gelsolin-null dermal fibroblasts reverts both the ruffling response, and Rac expression to normal. These results suggest that gelsolin is an essential effector of the Rac-mediated actin dynamics, acting downstream of the Rac recruitment to the
membrane. In order to understand EGF-mediated wound healing, it is crucial to study the underlying mechanisms of how Rac/cdc42 and gelsolin regulate the epidermis and fibroblast motility during wound healing.

**Fibroblast Growth Factor (FGF):** FGF-2 is modulated at sites of dermal tissue injury. To determine the role of FGF-2 in wound healing, the expression patterns of FGF-2 have been studied extensively in numerous experimental studies. For example, FGF-2 activity is detectable in wound fluids from both full- and partial-thickness wounds. During mouse skin wound repair, the FGF-2 protein or mRNA is localized to the basal layer keratinocytes and hair bulbs at the wound edge and in the reepithelialized area. The therapeutic effects of FGF-2 on wound healing have been examined in order to study further the functional role of FGF-2.

FGF-2 is involved in angiogenesis, extracellular matrix accumulation, and stimulates collagenolysis. FGF-2 has also been shown to increase the rate of epithelialization in excisional pig wounds and in healing-impaired diabetic mice. Fibroblasts seeded in an FGF-2-coated collagen I sponge matrix facilitate early dermal and epidermal wound healing. FGF-2 encapsulated in red blood cell ghosts also accelerates incisional wound healing. In a Phase I clinical trial, FGF-2 was applied to pressure sores in paraplegics. The healing rate among patients given the highest dose of FGF-2 was increased. However, a larger clinical trial with FGF-2 did not demonstrate a beneficial effect. This may be, at least in part, due to the fact that FGFs cannot activate their surface receptors without the cooperation of accessory molecules. FGF-2 binds to FGF receptors monovalently. Therefore, it is unable to promote either receptor dimerization or tyrosine kinase activation. Oligomerization of FGF molecules is mediated via multimeric interactions with soluble or membrane-attached heparin sulfate proteoglycans. This allows FGF to induce FGF receptor dimerization and tyrosine kinase activation. Indeed, in intact cells, heparin sulfate proteoglycans, are required for FGF stimulation of FGF receptor dimerization, tyrosine kinase activation, and signaling via the FGF receptors. While these studies provide fruitful information, further studies are needed to understand the molecular mechanisms of how FGF-2 enhances wound healing.

**Cytokine signaling in wound healing**

**Transforming Growth Factor-β (TGF-β):** TGF-β has been shown to enhance wound healing in numerous experimental studies. Systemic administration of TGF-β1 as early as 24 hours prior to wounding could accelerate the repair of cutaneous wounds. These results suggest that TGF-β can prime cells for increased responsiveness to factors released at the wound site, and that such signals can persist for as long as 24 hours.

To determine how TGF-β is secreted upon injury, the expression patterns of endogenous TGF-β have been studied. At the time of injury, latent TGF-β1 is released from the degranulating platelets into the wound bed as a bolus. Subsequently, injury-induced expression of immediate-early genes contributes to the transcriptional activation and autoinductive pathways of TGF-β1 expression, resulting in elevated levels of expression of endogenous TGF-β1 that persist over a protracted period. Since large stores of latent TGF-β are localized to the pericellular matrix, the proteolytic environment characteristic of the early stages of wound healing might also serve to release TGF-β locally from the extracellular matrix. Thus, the levels of TGF-β in the wound fluid remain elevated for up to 14 days, with peak levels 7 to 9 days following implantation of the wire mesh Hunter-Schilling chambers in the backs of the rats.

Immunohistochemical studies have shown that TGF-β isoforms are expressed in unique patterns following a wound. However, regardless of which TGF-β isoform is applied exogenously to a wound, only TGF-β1 is induced endogenously, and not TGF-β2 or TGF-β3. The expression patterns of the TGF-β isoforms in human skin suggests differences between human and animal models. TGF-β3 mRNA and its protein are prominently expressed in human derma, whereas little expression of TGF-β2 and TGF-β3 was observed in mice. TGF-β1 mRNA expression was also observed at the reepithelialization front of acute wounds. The TGF-β type II receptor was also expressed in the epidermis with stronger expression in the superficial, more differentiated layers. In mouse embryos, TGF-β1 mRNA is expressed transiently and in low levels after injury, but it is present in high levels for the duration of healing at the adult wound site. Delivery of antibodies that neutralize TGF-β1 and TGF-β2 at the time of wounding reduces scarring in the incisional
TGF-β3 has similar biological activities to those of TGF-β1 and -β2. However, the exogenous addition of TGF-β3 was recently reported to reduce scar formation in the rat incisional model. Furthermore, TGF-β3 down-regulates the other TGF-β isoforms. These results suggest that a balance among the TGF-β isoforms is critical in wound healing. Compared to this normal injury, the TGF-β isoforms are expressed differently in the fibroblasts derived from keloids. Further work is required in order to understand the potentially important differences in the biologic activities of the TGF-β isoforms in both normal and pathologic repair.

Molecular mechanisms of chronic wound keloid

Chronic wound keloid: Keloids are benign skin "tumors" caused by minor or severe skin injury. Excessive scar formation after a trauma or surgical injury can produce devastating chronic wounds such as body disfigurement and organ dysfunction. The lesions extend beyond the boundaries of the original wound, and manifest predominantly in pigmented individuals including Asians, Africans and Hispanics. The pathogenetic mechanisms underlying keloids formation continue to be elusive, though much of the focus has recently been aimed at elucidating the biomolecular pathways responsible for the excessive extracellular matrix accumulation. In this review, the recent progress made in understanding the molecular mechanisms of keloid scar formation and other chronic wounds will be discussed. These ongoing studies are aimed at providing a basis for molecular and cellular biology-based wound tissue engineering combined with well-controlled timing, efficacy and optimal dosage. These approaches will likely provide new therapies that are more effective in patients with fibroproliferative diseases of the skin and other tissues. Keloids appear as scar that grows beyond the confines of the original wound. Keloids may arise immediately after injury or years later. Typical keloid treatment consists of intra-lesional corticosteroid injections, used individually or in combination with surgery. Still, these lesions are often refractory to therapy; underscoring the need for further research. Discussions frequently compare keloids to hypertrophic scars. Their gross appearance is similar, though keloids grow beyond wound margins and rarely subside. However, they are histologically distinct. Keloids have stretched collagen bundles aligned in the epidermal plane, which is contrary to the relaxed, orderly appearance of collagen bundles in the normal skin or nodular, fine, randomly organized fibers of hypertrophic scars. The abundant collagen bundles are thick, tightly packed, acellular structures in the deep dermal portion of the keloid. However, the collagen does appear to be the same type as that in normal skin. Keloids contain relatively few cells at their center and no myofibroblasts. The keloid appears to be a homogenous mixture of cells, which behave differently depending on their location. For example, different growth properties and production of collagen I vary between regions within the keloid.

Tissue repair in keloids: Tissue repair is a complex cascade of events involving various cell types, extracellular matrix (ECM) components, cytokines and other soluble factors. A cascade of repair events begins with the formation of a fibrin-rich blood clot, and ends with the restructuring of newly synthesized scar tissue. Several vital sequential stages have been identified in the repair process, namely inflammation, fibroplasia, granulation tissue formation and scar maturation. The dynamic interaction and feedback control amongst the participating components govern the direction of the repair. Keloid fibroblasts demonstrate elevated gene expression for collagen, fibronectin, elastin and proteoglycan genes in vitro. Compared with normal dermal fibroblasts, keloid fibroblasts show aberrant responses to metabolic modulators, implicating their roles in the pathogenesis of keloid formation.

In vitro studies have identified many of the components that interact during wound healing. It appears that cellular gene expression is controlled through the adhesive interaction of connective tissue cells with the surrounding ECM. Many of these interactions are mediated through cell adhesion receptors called integrins. Integrin receptor expression is regulated by cytokines in an autocrine and paracrine manner. Indeed, proteolytic degradation of ECM is also an essential feature of tissue repair and the remodeling processes. Serine proteinases, including the plasminogen activator (PA) and the matrix metalloproteinases (MMPs), are ECM-degrading enzymes that provide a lytic cascade for ECM remodeling. The major function of the PA is conversion of plasminogen into plasmin. Plasmin is a fibrinolytic enzyme that breaks down the ECM protein and also converts procollagenase into its active collagenase.
form. Thus, the PA initiates the proteinase cascade, amplifying the proteolytic activity. In turn, plasmin activates the transforming growth factor-\(\beta\) (TGF-\(\beta\)) by releasing it from its latency-associated protein. TGF-\(\beta\) then acts on its target molecules thereby regulating plasminogen activator inhibitor 1 (PAI-1), MMPs, the tissue inhibitor of metalloproteinases-1 (TIMP-1) and the genes encoding the ECM components and their integrin receptors. It appears that all these interactions govern wound repair with a finely controlled equilibrium between matrix synthesis and degradation. Controversy still exists as to whether keloids are caused by increased collagen production, decreased degradation or both. Prolyl hydroxylase, the rate limiting enzyme in collagen synthesis, is upregulated in keloids. The measurement of \(^{14}\)C incorporation into hydroxyproline indicates an initial upregulation of collagen synthesis followed by a return to the baseline over several years. In contrast, other studies have shown an increase in the proteinase inhibitors alpha I antitrypsin, alpha II macroglobulin, PAI-1 and a decrease in the urokinase plasminogen activator (uPA) activities. Increased PAI-1 expression at both the mRNA and protein levels is unique to keloid fibroblasts. Increased PAI-1, for example, subsequently reduces plasmin stimulated collagenase production and plasmin activity. As a result, keloid fibroblasts exhibit a decreased capacity for fibrinolysis and, therefore, fibrin clot degradation. Although poorly understood, the elevated PAI-1 levels by the keloid fibroblasts may have significant consequences for the repair steps that follow fibrin clot removal. Indeed, higher numbers of proliferating fibroblasts are detected at the periphery of keloid lesions. In particular, the center of the keloid lesions has no proliferating cells. Finally, the multiple growth curves produced from in vitro cultures illustrated a lack of difference between the normal and keloid fibroblast growth kinetics.

**Signal transduction in chronic wound keloid:** The transforming growth factor-\(\beta\) (TGF-\(\beta\)) has been implicated in keloid pathogenesis. TGF-\(\beta\) increases the production of ECM elements, such as fibronectin and collagen, and upregulates cellular expression of the matrix receptor integrin. TGF-\(\beta\) mRNA and its related protein is associated with excessive collagen synthesis and ECM accumulation in the keloids. Keloid fibroblasts exhibit an altered response to the exogenous addition of TGF-\(\beta\). In addition, keloids exhibit an increase in fibronectin synthesis and also an increase in DNA and collagen synthesis. The three TGF-\(\beta\) isoforms identified in mammals (TGF-\(\beta\)1, \(\beta\)2, and \(\beta\)3) are thought to have different biological activities in wound healing. TGF-\(\beta\)1 and \(\beta\)2 are believed to promote fibrosis and scar formation, while depending on the system, TGF-\(\beta\)3 has been shown to either promote or retard scar formation. Recently, it was found that TGF-\(\beta\)1 and \(\beta\)2 proteins are highly expressed in keloids compared to normal human dermal fibroblasts. In contrast, the expression of the TGF-\(\beta\)3 protein was comparable in both normal and keloid cell lines. The mechanism of TGF-\(\beta\) receptor signaling has been intensively studied in order to understand TGF-\(\beta\)-mediated cellular responses. TGF-\(\beta\) stimulates the expression of ECM. Keloid fibroblasts respond to TGF-\(\beta\) by further increasing their already augmented rate of collagen synthesis, a phenomenon not detected in the fibroblasts of normal scar tissue. Keloid fibroblasts showed an altered response or the unique sensitivity of the keloid fibroblasts to TGF-\(\beta\) might reflect a change that occurs at the receptor level or in postreceptor signaling.

Many research groups are actively pursuing antagonists to the TGF-\(\beta\) that regulates the phenotypes of connective tissue cells during repair. Its purpose is to attenuate or regulate the excessive cell proliferation and synthesis and contract the ECM during repair by scar fibroblasts. Approaches taken to antagonize TGF-\(\beta\)-stimulated fibrosis include the use of neutralizing anti-TGF-\(\beta\) antibodies, soluble TGF-\(\beta\) type II receptors, the naturally occurring TGF-\(\beta\)-binding proteoglycan decorin and mannose 6-phosphate, which is an antagonist of TGF-\(\beta\) activation. In addition to TGF-\(\beta\), several other cytokines and growth factors have been implicated in keloid pathogenesis including the epidermal growth factor (EGF), the fibroblast growth factor (FGF), and the platelet derived growth factor (PDGF). The release and activation of the growth factors during the inflammatory phase of healing are prerequisites for subsequent processes, including angiogenesis, re-epithelialization, recruitment and proliferation of fibroblasts, and matrix deposition. Chemoattractants and mitogens, such as heparin, the fibroblast growth factor (FGF), interleukin-8 (IL-8), and the insulin-like growth factor-1 (IGF-1) stimulate angiogenesis. Wound re-epithelialization occurs following the migration of epithelial
cells from the wound margin and the epidermal appendages. EGF, TGF-alpha, the vaccinia growth factor, and IGF-1 enhance this process.\textsuperscript{10,105} Fibroblast recruitment, proliferation, and production of ECM are influenced predominantly by the fibrogenic growth factors PDGF, IGF-1, and TGF-\(\beta\), as well as the basic fibroblast growth factor.\textsuperscript{86} These fibrogenic growth factors upregulate ECM protein production, increase the proliferation and/or migration rate of fibroblasts, and inhibit the production of proteases required to maintain the balance between production and degradation.\textsuperscript{102} PDGF and CTGF have been implicated in fibrosis and are targets for blocking fibrosis.\textsuperscript{106} Cytokines such as interleukin 1, the tumor necrosis factor alpha and both interferon gamma and alpha, which suppress \(t\) collagen synthesis, have been used as antifibrotic agents.\textsuperscript{107} Despite the recent advancement in the therapeutic design for fibroproliferative disorders, further studies are still needed required to establish efficacy, timing and optimal dosage of these potential agents. Further investigation into the proper temporal and spatial expression of these agents during repair is necessary so as to properly understand the mechanism of normal healing and the treatment of pathological scarring.

\textbf{FUTURE PROSPECTS}

There is a large volume of knowledge regarding the role of various growth factors in wound healing.\textsuperscript{10,65,103} However, further experimental studies on the clinical use of growth factors in order to generate new tissue, to accelerate neovessel formation in ischemic tissue, and to promote tissue repair are still required. One of difficulties in studying the wound repair mechanisms is a redundancy and cross talk in the biology.\textsuperscript{10,33} Most repair signals most likely control more than a single cellular activity, and most of the cell activities are a response to a summation of signals. The redundancy of the multiple signals is becoming more apparent through studies using transgenic mice.\textsuperscript{108} The candidate genes thought to play a role in wound healing may also be important in normal development that a full gene knockout is lethal to the embryo. Nonetheless, both interbreeding of knockout mice and the careful design of transgenic mice with gene knockout, or dominant-negative receptor constructs with tissue-specific promoters should provide a wealth of further insight.\textsuperscript{103,108}

Growth factors are extremely valuable tools in our attempts to understand the mechanisms that modulate various cellular activities. Their specificity to particular cells and their ability to maintain adequate pharmacological levels is essential for successful repair, particularly in view of the proven effects that these compounds have on various cells and the dose dependence of their response. It will be interesting to examine the effects of growth factor-combination therapy on wound healing in the near future by taking advantage of both redundancy and cross talk by growth factor signaling.\textsuperscript{109} Clinical trials must focus on targeting growth factors for specific types of impaired healing in order to be cost effective. Pharmacological doses of growth factors can be delivered in an extracellular matrix molecule carriers that would promote the influx of the necessary cells into the wound. When targeted for specific problem wounds, this approach has the potential for making significant clinical improvements in wound healing.\textsuperscript{110} Difficulty in the treatment of keloids arises from the complexity of the molecular and cellular biology of keloids. Increased understanding at this level will lead to the development of new therapies. Control of the fibrogenic growth factor effects by monoclonal antibody techniques and control of receptor antagonists and through the development of antisense oligonucleotide therapy offers substantial potential. Appreciation of the immunologic response to injury and regulation of wound healing by the immune system will allow specific growth factor therapy to provide potential downregulatory signals, which some but not all individuals possess after wounding, thereby modifying the whole-body response to injury. Finally, with intense pursuit of skin replacements and the enhanced understanding of the role that the dermis has in controlling scar contracture and hypertrophy, skin replacement will likely provide new therapies previously unavailable for patients with fibroproliferative skin disease and other tissues. The complex nature of the repair process and the lack of proper \textit{in vitro} and \textit{in vivo} animals models for scar formation have hindered progress in revealing the mechanisms of pathologic scar formation. Recent \textit{in vitro} culture studies have the advantages of investigating certain hypotheses under defined systems. These include physiological relevance to \textit{in vivo} conditions and well-defined and controllable parameters of the cell type and number, and the

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matrix type and concentration. Experimental reproducibility and a microwell format provides a feasibility for pharmacological studies. Therefore the system is well suited for some creative designs in the study of the mechanism under both normal and abnormal healing processes.

Transgenic and knockout animals also provide a new approach to the investigation of gene function in vivo. 108. Plasminogen-deficient mice provide definitive proof of the involvement of plasmin in wound repair. 11 These mice exhibit impaired skin wound healing, abnormal keratinocyte migration and the protrusion of excessive granular tissue in the middle of the wound, resembling a raised scar. Furthermore, a knockout of the keratinocyte growth factor gene shows the defects in wound healing. 112

The mechanisms of fetal wound repair have attracted much attention in recent years, due to the observation that fetal wounds heal without scar formation. Fetal skin contains abundant hyaluronic acid and does not become inflamed during repair. Nevertheless, a key to fetal scarless healing seems to reside in TGF-β expression. Whereas adult wound sites contain high and persistent TGF-β1 levels, TGF-β1 is fleetingly expressed at low levels in fetal wounds. 33 Therefore, understanding the mechanisms of TGF-β expression and the mode of action on cell proliferation/differentiation and ECM production/remodeling might provide us with the ultimate handle on the control of excess scarring in adult wounds.

The successful application of nuclear transfer techniques to a range of mammalian species has brought the possibility of stem cell-based human therapies. We can produce pluripotent stem cells that carry the nuclear genome of the patient and then induce them to differentiate into replacement cells, such as cardiomyocytes to replace damaged heart tissue or insulin-producing beta cells for patients with diabetes. 1-3 This approach would eliminate the critical problem of immune incompatibility. The next task will be reconstituting the cells into more complex tissues and organs in vitro. Both committed stem and progenitor cells have also been isolated from various adult tissues, including hematopoietic stem cells, neural stem cells, hair follicle stem cells, mesenchymal stem cells and endothelial progenitor cells. These adult stem cells have several advantages when compared to embryonic stem cells in terms of their practical therapeutic application for tissue regeneration. 1,4-8 The application of promising gene therapies using adult stem and progenitor cells in terms of modifying stem cell potency, altering organ property, accelerating regeneration and forming expressionional organization is certain to occur in the near future.

The next decade will be exciting as we attempt to determine whether we can induce the successful healing adult wounds, while at the same time decrease or eliminate fibrosis and scarring, by utilizing stem cell-based tissue engineering technologies.

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