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Signal Transduction in Wound Pharmacology

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Growth factors such as TGF-beta, PDGF and FGF are thought to play important roles in wound healing. However, their biological activity and signal transduction during wound repair remain poorly understood. Growth factors are often ligands for receptor tyrosine kinase and receptor serine/threonine kinases. With recent advances in signal transduction by receptor kinases, we are beginning to understand the underlying mechanism of how growth factors may regulate cutaneous wound repair. In this paper, we will describe the pharmacological effects of growth factors on wound healing, and discuss the potential underlying signaling mechanisms. Thus, we hope to provide the basis for designing more specific therapeutics for wound healing in the near future.

Key words : Growth factors, Receptor kinases, Signal transduction, Wound healing, Skin

INTRODUCTION

Wound healing is a complex cellular process, including inflammation, extracellular matrix synthesis, collagen deposition, angiogenesis, and reepithelialization (Clark, 1993; Martin, 1997). 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 (Mustoe *et al.*, 1987). Growth factors discovered in the processes of wound repair have opened the door to new therapeutics to manipulate cutaneous repair (Deuel *et al.*, 1991; Pierce & Mustoe, 1995).

Growth factors such as epidermal growth factor (EGF), platelet derived growth factor (PDGF) and fibroblast growth factor (FGF) are the ligands for receptor tyrosine kinases. Receptor tyrosine kinases exhibit similar molecular structure and are activated by a common mechanism. The mechanism for lignd-induced to the extracellular domain induces receptor dimerization, which leads to activation of the catalytic domain of protein tyrosine kinase and also leads to tyrosine autophosphorylation. (Schlessinger & Ullrich, 1992; Schlessinger, 1997). Phosphorylation of tyrosines within the catalytic domain is essential for maintaining the tyrosine kinase in an active state, while phosphorylation of tyrosine residues that are located in noncatalytic regions leads to generation of docking sites for SH2 (Src homology 2) and PTB (phosphotyrosine binding) domains of signaling molecules (Pawson, 1995; Kim, 1996). A variety of signaling proteins are directly recruited by activated receptor while other signaling molecules are activated by tyrosine phosphorylation (Schlessinger & Ullrich, 1992; Kim, 1996). A variety of signaling proteins are directly recruited by activated receptors while other signaling molecules are activated by tyrosine phosphorylation (Schlessinger & Ullrich, 1992; Kim, 1996). In many cases, the ligands of growth factor receptors 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-beta signaling requires a heteromeric assembly of its two serine-threonine kinase receptors, designated RI and RII, respectively (Wrana & Pawson, 1997). A recent model illustrating 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 (Massague, 1996, 1997). The discovery of SMAD (mammalian homolog of Drosophila Mad gene) proteins has allowed the delineation of a mechanism by which TGF-beta and related growth factors convey their signals from membrane receptors into the nucleus. SMADs are directly phosphorylated and activated by the receptors and then form heteromeric SMAD-SMAD complexes that move into the nucleus where they orchestrate

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transcriptional responses (Massague, 1997; Kretzschmar & Massague, 1998). Different modes of SMAD interactions are regulated by phosphorylation. The SMAD domains that mediate SMAD interactions, binding to DNA, and transcriptional activation, have been defined. The recent discovery of antagonistic SMADs and regulatory crosstalk with Ras/MAP-kinase pathways add to our rapidly expanding knowledge of the major regulatory network (Kretzschmar & Massague, 1998). Defects in expression of either receptors would contribute to the loss of response to exogenous and endogenous TGF-beta. Interestingly, in some fibrotic diseases, the expression levels of SMAD molecules are quite different, when compared to normal human skin (Kim *et al.*, unpublished data). Further experiments will be necessary to elucidate whether SMAD molecules play a role in normal wound repair and fibrotic skin diseases.

Transforming growth factor-beta (TGF-beta)

It has been well established that endogenous and exogenous TGF-beta enhances wound healing in numerous experimental studies (Martin, 1992; Roberts & Sporn, 1993). Systemic administration of TGF-beta 1 as early as 24 hours prior to wounding led to accelerated repair of cutaneous wounds (Beack, 1993). These results suggest that TGF-beta 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-beta is secreted upon injury, expression patterns of endogenous TGF-beta have been studied. At the time of injury, latent TGF-beta 1 is released from degranulating platelets into the wound bed as a bolus. Subsequently, injury-induced expression of immediated-early genes contributes to the transcriptional activation and autoinductive pathways of TGF-beta 1 that persist over a protracted period. Since large stores of latent TGF-beta are localized to pericellular matrix, the proteolytic environment characteristic of the early stages of wound healing might also serve to release TGF-beta locally from the extracellular matrix. Thus, the levels of TGF-beta in wound fluid remain elevated for up to 14 days, with peak levels on days 7 to 9 following implantation of wire mesh Hung-Schilling chambers in the back of rats, at the time of maximum fibroblast proliferation and collagen synthesis (Cromack *et al.*, 1987). Immunohistochemical studies showed that TGF-beta isoforms are expressed in unique patterns following wounding (Levine *et al.*, 1993). Expression patterns of the TGF-beta isoforms in human skin suggest that there may be differences compared to the animal models (Roberts & Sporn, 1996). Whereas little expression of TGF-beta 2 and -3 was seen in mice, TGF-beta 3 mRNA and protein are prominantly expressed in human dermis (Schmid *et al.*, 1993a). TGF-beta 1mRNA expression was seen at the reepithelialization front of acute wounds. TGF-beta type II receptor was also expressed in the epidermis with stronger expression in the superfacial, more differentiated layers (Schmid *et al.*, 1993a). In the mouse embryo, TGF-beta 1 mRNA is expressed transiently and at low levels after injury (Martin *et al.*, 1993), but it is present at high levels for the duration of healing at the adult wound site (Frank *et al.*, 1996). Delivery of antibodies that neutralize TGF-beta 1 and beta 2 at the time of wounding reduced scarring in the incisional rat model (Shah *et al.*, 1992). TGF-beta 3 has similar biological activities to those of TGF-beta 1 and 2. However, the exogenous addition of TGF-beta 3 was recently reported to reduce scar formation in the rat incisional model (Shah *et al.*, 1995). These results suggest that a balance among the TGF-beta isoforms is critical in wound healing. Further work is required to understand the potentially important difference in the biologic activities of the TGF-beta isoforms in normal and pathologic repair.

Epidermal growth factor (EGF)

Topical application of EGF in human cutaneous wound repair showed a modest acceleration in the rate of skin resurfacing in partial thickness, donor site wounds (Brown et al., 1989). The modest effect of topical EGF in wounds is a reflection of the complex interactions of cells, growth factors/cytokines, type of wound, and individual patient differences. The clinical challenge remains of how to target the EGF receptor pathways so that acute and chronic human cutaneous wounds achieve satisfactory healing despite the presence of underlying disease state such as diabetes or other adverse wound-healing circumstances (Nanney & King, 1996). It is difficult in biological trials to mimic *in vitro* models of EGF receptor signal transduction pathways that provide clear-cut results. These difficulties in defining a precise function for the EGF receptor or any other cytokine *in vivo* are related to a number of variables such as age, anatomical site, proliferative state, degree of differentiation, preexisting cutaneous and systemic abnormalities, temporal intervention after injury, type of injury, and other undefined genetic and environmental factors (Nanney & King, 1996). To date, the regulation of EGF receptor expression has been examined by therapeutic trials, immunohistochemistry, and *in situ* hybridization.

In numerous therapeutic studies, EGF showed significant enhancement of wound healing (Mustoe *et al.*, 1991). EGF stimulates epidermal repair in animal excisional and thermal injury models, and may also stimulate dermal repair (Schultz *et al.*, 1987). EGF enhances reepithelialization in burn wounds on the

backs of pigs (McGee *et al.*, 1988) and appears to be effective in accelerating repair in chronic ulcers (Brown *et al.*, 1991; Falanga *et al.*, 1992).

To gain insight into the participation of EGF receptor and its ligands in wound repair, the expression of EGF receptor was examined in normal neonatal and adult skin by immunohistochemistry and *in situ* hybridization. EGF receptor has been localized throughout all nucleated layers of the neonatal epidermis. However, in adult dermis, EGF receptor is spatially positioned only on basal layer keratinocytes, cells that are of prime importance for the resurfacing of human partial-thickness wounds. The presence or absence of EGF receptor on specific keratinocyte populations in the epidermis and within epidermal appendages implies regulatory and biological significance for this cytokine signaling pathway. Since skin appendages are situated deep in the dermis and subcutaneous tissues, this population of keratinocytes is better protected from the environment and, therefore, serves as a ready source of cells to replace the overlying epidermis lost during wounding. During wound-healing, EGF receptor-mediated events appear to play a vital role in reforming the epidermal permeability barrier, keratinocyte differentiation, keratinocyte proliferation, and keratinocyte migration and adhesion (Nanney & King, 1996). Injections of EGF into neonatal mice showed that EGF could affect epithelial structures by modulating the normal developmental process (Nanney & King, 1996). However, the underlying mechanisms by which EGF regulates repair remain undefined.

To further investigate the functional role of EGF in wound healing, EGF receptor signal transduction mechanisms have been extensively studied (Schlessinger & Ullrich, 1992; Pawson, 1995). In the EGF receptor signal transduction pathway, we are now able to trace molecules relaying signals from receptor to nucleus, such as Grb2 (Schlessinger, 1997). More recently, much attention has been paid to protein tyrosine phosphatases (PTPases). Identification of a large family of PTPases has lead to the study of interactions of tyrosine kinase such as EGF receptor with PTPases (Fischer *et al.*, 1991; Kim, 1996). PTPases have been localized in normal human skin, but have not been examined in would healing (Clark, 1996). Since stimulatory signals through tyrosine kinase receptors such as EGF receptor must be attenuated with regulatory molecules such as the PTPases, it will be fruitful to study the potential role of PTPases in wound healing. Until recently, it was not clear how extracellular signals might affect cell motility involved in wound healing, but it is now known that EGF ligand binding to receptor activates the signaling molecule MEK, and that MEK selectively interacts with the small guanosine triphosphatase (GTPase) Rac/Cdc42 (Fanger *et al.*, 1997; Frost *et al.*, 1997). When Rac is activated in 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 (Martin, 1997). Interestingly, recent studies showed that gelsolin, crucial for organization of actin filament, is a downstream effector of Rac for fibroblast motility (Azuma *et al.*, 1998). Compared to wild type dermal fibroblasts, gelsolin in gelsolin-null dermal fibroblasts reverts the ruffling response to EGF. Stable expression of gelsolin in gelsolin-null dermal fibroblasts reverts the ruffling response, and Rac expression to normal. These results suggest that gelsolin is an essential effector of Rac-mediated actin dynamics, acting downstream of Rac recruitment to the membrane. To understand EGF-mediated wound healing, it is crucial to study underlying mechanisms how Rac/cdc42 and gelsolin regulate epidermis and fibroblast motility during wound healing.

Platelet-derived growth factor (PDGF)

It has been shown that PDGF is potent stimulator for wound healing in animal experiments and in human clinical trials (Helding & Westermark, 1996). For example, PDGF enhances angiogenesis and epithelialization in excisional wound models (Pierce et al., 1992), and increases the breaking strength of incisional wounds in both normal and impaired healing models (Pierce *et al.*, 1989). PDGF also accelerates the deposition of 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.

PDGF receptor signal transduction mechanisms have been extensively studied. PDGF induces tyrosine phosphorylation of the PDGF receptor and numerous other intracellular proteins (Schlessinger & Ullrich, 1992; Li *et al.*, 1994). PDGF receptor mediates fibroblast chemotaxis, proliferation, and induction of extracellular matrix and metalloproteinases, which are required for wound remodeling (Deuel *et al.*, 1991; Bennett & Schultz, 1993). Until recently, it was not known which molecules are involved in PDGF-mediated wound healing. However, we now know that PDGF activates the small GTPases Rac in fibroblasts (Nobes & Hall, 1995). Gelsolin, as described earlier, is a downstream effector of Rac (Hartwig *et al.*, 1995). So it is likely that Rac may be one of the key molecular switches responsible for the onset of PDGF-mediated fibroblast migration into a wound. Further studies are required for identification of the PDGF receptor-secondary signaling molecules to fully understand the mechanisms of PDGF-mediated wound repair. It is also necessary

to determine the specificity of tyrosine kinases in accelerating wound healing. PDGF may be an important future clinical tool, particularly for the stimulation of soft tissue repair in patients with impaired capacity for wound healing. Much work remains, however, to optimize dose, methods of administration, choice of PDGF isoform, etc (Clark, 1996). Moreover, critical comparisons with other growth factors should be performed in order to select the best factor, or combinations of factors, for different types of wounds.

Fibroblast growth factor (FGF)

It is well established that among FGF family members, FGF-2 is present and modulated at sites of dermal tissue injury (Abraham & Klagsbrun, 1996). To begin to determine the role of FGF-2 in wound healing, 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 (Chen *et al.*, 1992; Werner *et al.*, 1992). During the mouse skin wound healing, FGF-2 protein or mRNA is localized in the basal layer keratinocytes and hair bulbs at the wound edge and in the reepithelialized area (Kurita *et al.*, 1992). To further study the functional role of FGF-2, therapeutic effects of FGF-2 on would healing have been examined.

FGF-2 is involved in angiogenesis, extracellular matrix accumulation, and also stimulates collagenolysis. FGF-2 has also been shown to increase the rate of epithelialization in excisional pig wounds and in healing-impaired diabetic mice (Mustoe *et al.*, 1991; Pierce *et al.*, 1992; Tsuboi *et al.*, 1992; Legrand *et al.*, 1993). Fibroblasts seeded in an FGF-2-coated collagen I sponges matrix facilitate early dermal and epidermal wound healing (Marks *et al.*, 1991). FGF-2 encapsulated in red blood cell ghosts also accelerates incisional wound healing (Slavin *et al.*, 1992). In a Phase I clinical trial, FGF-2 was applied to pressure sore in paraplegics and the healing rate among patients at the highest dose of FGF-2 was increased (Robson *et al.*, 1992). However, a larger clinical trial with FGF-2 did not demonstrate a beneficial effect of FGF-2. This may be, at least in part, due to the fact that FGFs can not activate their surface receptors without the cooperation of accessory molecules. FGF-2 binds to FGF receptors monovalently, and is, therefore, unable to promote receptor dimerization and tyrosine kinase activation (Schlessinger *et al.*, 1995). Oligomerization of FGF molecules is mediated via multimeric interactions with soluble or membrane-attached heparin sulfate proteoglycans, allowing 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 FGF receptors (Spivak-Kroizman *et al.*, 1994). While these studies investigating FGF signal transduction provide fruitful information, further studies are required to understand the molecular mechanisms of how FGF-2 enhances wound healing.

FUTURE PROSPECTS

There has been an accumulation of knowledge about the role of growth factors in wound healing (Pierce & Mustoe, 1995), however further experimental studies are still required for the clinical use of growth factors to generate new tissue, to accelerate neovessel formation in ischemic tissue, and to promote repair. One of the difficulties in studying wound repair mechanisms is redundancy and cross-talk in the biology (Clark, 1996; Martin, 1997). Most repair signals probably control more than one cellular activity, and most of cell activities are a response to a a summation of signals. The redundancy of these multiple signals is becoming more apparent through the study of transgenic mice. Candidate genes thought to play a role in wound healing may also be important in normal development such that a homozygous gene knockout is lethal to the embryo. Nonetheless, interbreeding of knockout mice and the careful design of transgenic mice with gene knockouts or dominant-negative receptor constructs with tissue-specific promoters will provide a wealth of further insight (Pierce & Mustoe, 1995).

Growth factors are extremely valuable tools in our attempts to understand the mechanisms that modulate cellular activities. Targeting of growth factors to specific cells and maintaining adequate physiological levels may be essential for successful repair. Taking advantage of redundancy and cross talk by growth factor signaling, it will be interesting to examine the effects of growth factor-combination therapy on wound healing in the near future (Nimni, 1997). To be cost effective, clinical trials must focus on targeting growth factors for specific types of impaired healing. Pharmacological doses of growth factors can be delivered in extracellular matrix molecule carriers that would promote the influx of necessary cells into the wound. When targeted for specific problem wounds, this approach has the potential for making significant clinical improvements in wound healing (Greenhalgh, 1996). The next few years will be exciting for wound pharmacology, as we test to see whether we can induce adult wounds to heal successfully, while at the same time decreasing or eliminating fibrosis and scarring.

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