Molecular biology of wound healing

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ABSTRACT

Wound healing is a dynamic process that involves the integrated action of a number of cell types, the extra cellular matrix, and soluble mediators termed cytokines.In recent years considerable advances have been made in the research, knowledge, and understanding of growth factors. Growth factors are, in essence, proteins that communicate activities to cells. Their function is dependent on the receptor site they attach to. Growth factors were initially named for the type of response generated by them, but newer research has shown that many of these cells may accomplish many different types of response. A growth factor's role in wound repair is a critical component of the successful resolution of a wound. Growth factors help regulate many of the activities involved in healing. The role and function of growth factor is an evolving area of science and offers the potential for treatment alternatives in the future.

KEY WORDS: Cytokines, growth factor, wound healing

The process of wound healing begins immediately following injury wound repair process requires close control of degradative and regenerative processes involving numerous cell types and complex interactions between multiple biochemical cascades. Growth factors released in the traumatized area promote cell migration into the wound area (chemotaxis), stimulate the growth of epithelial cells, and fibroblasts (mitogenesis) initiate the formation of new blood vessels (angiogenesis) and stimulate matrix formation and remodeling of the affected region. Animal studies have shown that exogenously added growth factor can accelerate the normal healing process. Growth factors have been used successfully in humans to treat previously incurable wounds. The most intensively studied growth factors are epidermal growth factor (EGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), transforming growth factor (TGF)-α, and TGF-β. Each of these factors is currently the focus of intense commercial development.[1]

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Growth Factors in Wound Healing

Growth factors are classified as cytokines which are proteins that act as internal cellular signals to allow cells to communicate with one another. Growth factors are actually a subclass of cytokines that specifically stimulate the migration and proliferation of cells and synthesis of new tissue.

These cytokines range in weight from to 6 to 70 kd and they direct cellular activities when they are present in small quantities. Cytokines can regulate cellular activities and function via endocrine, paracrine, autocrine, and intracrine mechanisms.

In order for a particular cytokine to modulate a cellular activity, the target cell must have a receptor. Once receptor binding takes place, then a series of intracellular signals are activated and eventually results in a specific response.

Within healing wounds, various proteins have been identified that exhibit special properties important for repair. One family of proteins has been called growth factors even though their actions are not restricted to stimulation of growth. In fact, the terminology used to label growth factors is often misleading because the names were derived from tissues in which the factors were initially identified. An often cited example of the anachronistic manner in which growth factors are labeled is PDGF. PDGF was originally identified in the α granules of

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platelets, but since then has also been found in macrophages, endothelial cells, and smooth muscle cells. Many of these proteins are capable of both chemotactic and mitogenic functions. They are therefore important in determining both the sequence of cells in the "healing module" and their respective functional activities. Several growth factors for epithelial repair are identical to those responsible for hard tissue regeneration.

Growth Factor Receptors

Growth factors are believed to exert their specific effects through specific receptors present on the surfaces of target cells. Receptors may be categorized as direct catalytic receptors and G-protein–coupled receptors. The method of receptor–ligand binding and subsequent cell stimulation is unknown. However, several models describing potential mechanisms have been proposed. It is known that receptors are composed of three parts: a cytoplasmic region, a hydrophobic transmembrane region, and an extracellular ligand-binding domain. One model for the activation of direct catalytic receptors [Figure 1] suggests that occupation of a receptor site triggers intracellular reactions by the aggregation of separate receptors on the cell surface. Because receptors are connected to the intracellular domain through short chains of hydrophobic amino acids, a pathway is provided through which intracellular enzymes may be activated.[2] Phosphorylation of intracellular serine, threonine, or tyrosine residues by protein kinases (tyrosine kinase) has been associated with activation of target cells. These intracellular reactions induce conformational changes in the cell. The process by which extracellular physical activities are convened to intracellular chemical changes is referred to as signal transduction.

Activation of G-protein–coupled receptors involves GTP-binding proteins called G-proteins. These are present on the inner surfaces of target cells close to receptor sites. G-proteins affect a variety of intracellular activities including regulation of enzymes and ion channels within the cell membrane.

A third receptor–effector mechanism acts through a set of genes that form the cellular counterparts to viral oncogenes. These are known as proto-oncogenes, and transcription of two genes, *c-fos* and *c-myc*, has been reported shortly after exposure of cells to PDGF. The exact function of these genes is unknown, but they are believed to prepare the cell for DNA synthesis and division.

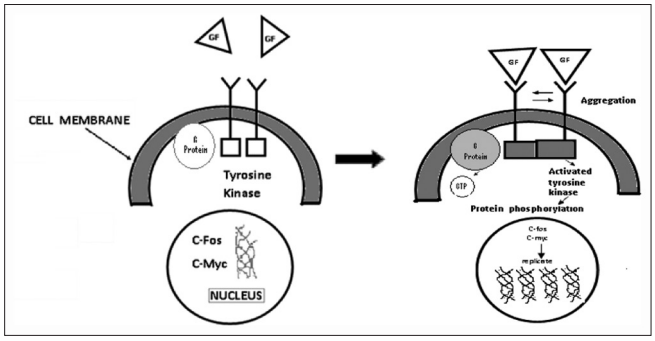

Figure 1: Growth factors (GF) with specific receptors

Growth Factor Transmission

Growth factors are ubiquitous throughout a wound and can be localized over the three phases of healing. Because their activity is affected by local concentration levels, the various modes of delivery from source to target become important. There are three ways in which growth factors are transported. Factors secreted into the blood and conveyed to a distant site use an endocrine mode of delivery.[3] Insulin-like factors I and II are examples of this. PDGF, TGF-α, and TGF-β exert their effects through a paracrine mechanism in which the secretory product of one cell acts directly on another. Cell to cell distances are therefore important for paracrine factors to act efficiently. Autocrine routes are also known and these help cells perform self-regulatory functions. TGF-β is an example of an autocrine factor whose secretory cell is both the source and target of its activity. Multiple methods of delivery are possible for certain factors with the use of paracrine, endocrine, or autocrine paths to modulate different functions through varying dose–response relationships [Figure 2].[4]

Classification of Growth Factors According to Temporal Activity

Throughout this description of wound healing, a recurrent feature has been the importance of timing for cells and enzymes to exert their influence. Growth factors fit into this scheme by the temporal nature of their activities. Depending on their period of activity, an alternative method of classifying growth factors has emerged. Factors that act on quiescent cells in the G_o phase of the cell cycle have been termed competence factors. Examples of these are PDGF and FGF. Other factors are termed progression factors and include the insulin-like growth factors (IGFs) and a macrophage-derived growth factor. These act later in the cell cycle during the S and G_2 phases. This separation of activity creates the impression that factors are limited to fixed stages of cell development, whereas in reality, changes in local conditions (e.g. factor concentration) allow growth factors to act on different periods of cellular growth, producing different effects.

Timing of activity may have significant pharmacologic relevance as various growth factor therapies are developed to aid wound repair. Studies are currently underway to investigate the benefits of multiple growth factor administration to optimize interactions with cells progressing through different phases of the cell cycle. Other investigations are focusing on

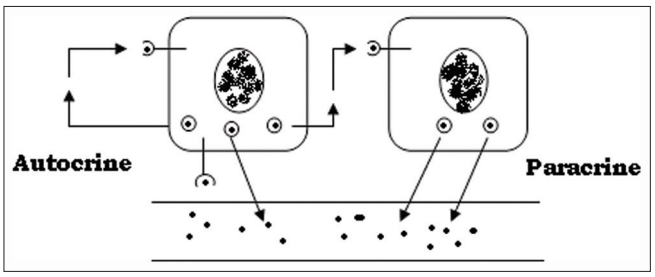

Figure 2: Autocrine and paracrine interactions

repeated dosing of the same growth factor at different periods. Appropriately timed growth factor combinations appear to successfully enhance wound repair. For instance, investigators have shown that combing PDGF and IGF-1 proved more beneficial for wound repair than either factor alone. Purportedly, PDGF promoted cell competence, whereas IGF-1 stimulated cell progression. This synergistic action helped amplify the respective healing processes.

Selected Growth Factors in Wound Healing

Several growth factors have been identified in wounds, and their functional roles have been investigated with both cellular and animal models. Although new activities are constantly being discovered for each of the factors, some of the more important features will be discussed.[5] A summary of the pertinent properties of several growth factors relevant to epithelial repair is presented in Table 1.

Epidermal growth factor and TGF-α

EGF (molecular weight 6 kDa) was first isolated from the submandibular gland of mice. It was shown to be a potent mitogen and maturation factor for epidermal cells.[6,7] Present in most body fluids and expressed at very low levels in normal tissues, EGF and TGF-α are closely related (35% homology) and possess very similar activities. Both have a molecular weight of 5700d and affect mesenchymal and epithelial cells. They are derived from transmembrane proteins and act through a paracrine mechanism on the EGF receptor. Wound macrophages contain significant amounts of TGF-α that add to the significance of this cell in the initial tissue response to injury. The main effect of TGF-α and EGF appears to be on granulation tissue development, with epidermal re-growth and modulation of angiogenesis being unique features of TGF-α activity.[4]

Table 1: Growth factors in wound healing

Growth factor	Source	Wound healing related functions
PDGF	Platelets, macrophages, endothelial cells, injured cells	Chemotaxis, fibroblast proliferation, collagenase production
TGF-β	Macrophages, platelets, neutrophils, lymphocytes, fibroblasts, epithelial and endothelial cells, injured cells	Fibroblast proliferation, chemotaxis, collagen metabolism
EGF	Plasma, platelets, macrophages, epithelial cells	Epithelial cell proliferation, granulation tissue formation
TGF-α	Activated macrophages, platelets, epithelial cells, injured cells	Epithelial cell proliferation, granulation tissue formation
KGF	Fibroblasts	Epithelial cell proliferation
IL-1	Macrophages	Fibroblast proliferation
FGF	Pituitary, macrophages, fibroblasts, endothelial cells	Fibroblast proliferation, matrix deposition, wound contraction angiogenesis
TNF-α	Macrophages, T lymphocytes	Fibroblast proliferation
IGF-1	Plasma, liver, fibroblasts	Synthesis of sulfated proteoglycans and collagen, fibroblast proliferation
IFNs	Lymphocytes, fibroblasts	Inhibition of fibroblast proliferation and collagen synthesis

Platelet-derived growth factor

PDGF (molecular weight 30 kDa) is a dimeric glycoprotein consisting of two chains (A and B). Three different isomers of PDG exist (AA, AB, and BB dimers), of which PDGF-AB is the most common.[8]

PDGF is stored in the α granules of platelets and released after activation of the platelets at sites of tissue injury. Macrophages, endothelial cells, vascular smooth muscle cells, and fibroblasts also express PDGF, which is one of the most potent competence factors present in wounds and which exerts effects during the first two phases of repair. It has been shown to be chemotactic for fibroblasts and monocytes as well as mitogenic for fibroblasts and vascular smooth muscle cells. The production of PDGF at wound sites is not constant, and increases in concentration have been correlated with augmented connective tissue formation. PDGF acts through paracrine and autocrine mechanisms that enable it to function not only as a stimulator of cellular activity but also in a homeostatic feedback fashion. Stimulation of the phosphorylation enzyme, tyrosine kinase, and increased transcription of the proto-oncogenes, *c-fos* and *c-myc*, have been observed with applications of PDGF.[9]

Transforming growth factor-β

TGF-β (molecular weight 25 kDa) is a dimeric glycoprotein comprising two identical chains originally isolated from the media of transformed cells *in vitro*. Three separate isoforms of the TGF-β superfamily exist in humans. They differ in their areas of distribution, but share similar, but not identical, functional activity. The TGF-β family of molecules is highly conserved across many species and plays an important role in embryogenesis and, in particular, in those areas of the embryo derived from neural crest mesenchyme. It is here that epithelial–mesenchymal interactions are of crucial importance in the development of the face and teeth.[10]

TGF-α and TGF-β were first isolated from tumors and named for their ability to transform normal cells into malignant phenotypes. TGF-β has been identified in a wide variety of cells including platelets, macrophages, bone cells, monocytes, lymphocytes, and platelets. Commensurate with its diverse origins, this factor has both mitogenic effects as well as regulatory functions over matrix production. TGF-β is chemotactic for fibroblasts and monocytes and is capable of stimulating or inhibiting fibroblasts. *In vivo* applications of TGF-β promote cellular influx into the wound and increase the synthesis of extracellular matrix proteins. In addition, TGF-β has been shown to stimulate angiogenesis through the induction of two monocyte-related ploypeptides, interleukin-I and tumor necrosis factor-α (TNF-α; macrophage-dependent angiogeneic activity).

Recently, recombinant DNA techniques have been applied to the study of TGF-β. The results indicate that several different proteins comprise the TGF-β superfamily. Two of these, TGF-β I and TGF-β 2, possess structural and chemical similarities to proteins of the bone morphogenetic protein group.

Basic fibroblast growth factor

FGFs (molecular weight 150 kd) are a family of homologous peptides, whose name was originally derived from their ability to stimulate fibroblast proliferation *in vitro*.[11]

Basic fibroblast growth factors (bFGFs) comprise a group of regulatory molecules with a high affinity for heparin. Release of basic fibroblast growth factors occurs due to the action of the enzyme heparinase found in platelets by dissolving the heparin binding. FGFs are found in many different tissues including endothelial cells, macrophages, and fibroblasts, and are both chemotactic toward endothelial cells and leukocytes as well as mitogenic for endothelial cells. As expected, bFGF plays a prominent role in angiogenesis, initiating release of basement membrane degrading enzymes that liberate endothelial cells before new vessel formation.

Tumor necrosis factor-α

Macrophages can stimulate angiogenesis by expressing TNF-α, a substance first associated with the necrosis and regression of certain solid tumors. This direct activity, as opposed to the indirect effects of PDGF, was originally assigned to macrophage-derived angiogenesis activity factors. Recent investigations have identified this factor to be the same as TNF-α.

Understanding of the events in wound healing provides a framework to comprehend the beneficial impact of some drugs and the negative aspects of other pharmacologic agents in wound healing. Drugs which benefit wound healing must be used at the appropriate time to have the beneficial effect. Cytokines are now being successfully used as beneficial topical agents in healing wounds.[12] Local factors such as infection, hypoxia, or foreign bodies are important in the healing wound and should be corrected or controlled.

References

1. Oijke P, Iwtha KK. Wound Healing. Nat Biotechnol 1989;7:793-8.
2. Wong EK, Holinger JO, Pinero GJ. Integrated processes responsible for soft tissue healing. Oral Surg Oral Med Oral Pathol 1996;82:475- 92.
3. Picarda SM, Schor SL. The structure and function of oral mucosa. Dent Update 1991;17:208-16.
4. Whitman M. Melton DA. Growth factors in early embryogenesis. Ann Rev Cell Biol 1989;5:93-117.
5. Rothe MJ, Falanga V. Growth factors and wound healing. Clin Dermatol 1992;9:533-59.
6. Cohen S. The stimulation of epidermal proliferation by a specific protein (EGF). Dev Biol 1965;12:394-6.
7. Ullrich A, Schlessinger J. Signal transduction by receptors with tyrosine kinase activity. Cell 1990;61:203-6.
8. Ross R. Raines EW, Bowen-Pope DF. The biology of platelet derived growth factor. Cell 1986;46:155-60.
9. Thomas DW, O'Neill ID, Harding KG. Shepherd JP. Cutaneous wound healing: A current perspective. Oral Maxillofac Surg 1995;53:442-7.
10. Rodiand KD, Muldoon LL, Magun BE. Cellular mechanisms of TGF-β action. J Invest Dermatol 1990;94:33.
11. Gospodarowicz D. Localisation of a fibroblast growth factor and its effect alone and with hydrocortisone on 3T3 cell growth. Nature 1974;249:123-7.
12. Karukonda SR, Flynn TC, Boh EE, McBurney EI, Russo GG, Millikan LE. The effects of drugs on wound healing: Part - 1. Int J Dermatol 2000;39:250-7.

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