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MOLECULAR PATHWAYS OF WOUND HEALING – A REVIEW

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ABSTRACT

Wounds healing in a very organized manner characterized by four distinct, but overlapping phases: hemostasis, inflammation, proliferation and remodeling. Wounds may be acute or chronic. These require various growth factors for proper healing and regeneration of the tissue. In addition to stimulating cell proliferation, growth factors may also have effects on locomotion, contractility, differentiation and angiogenesis. This review describes the major growth factors and biological processes involved in the normal healing.

KEYWORDS: wound healing, growth factors, cell proliferation, angiogenesis.

INTRODUCTION

In response to an injury, cellular and vascular responses by the host occur. The injury may be acute or chronic. Acute if the stimulus is severe but short lived and chronic, if the stimulus is persistent. Acute injury may lead to parenchymal cell death with intact tissue framework (superficial wounds) or parenchymal cell death with damaged tissue framework (deep wounds). Parenchymal cell death with intact tissue framework often heals by regeneration *i.e.* restitution of normal structure. Parenchymal cell death with damaged tissue framework mostly heals with scarring.[1] Persistent tissue damage in case of chronic injury heals with

fibrosis that mostly occur due to activation of macrophages and lymphocytes. These induce the formation of growth factors (PDGF, FGF, TGF-α), cytokines (TNF, IL-1, IL-4, IL-13) and decreased metalloproteinase activity. Growth factors cause proliferation of fibroblasts, endothelial cells and specialized fibrogenic cells. Cytokines cause increased collagen synthesis and decreased metalloproteinase activity leads to decreased collagen degradation. These all leads to fibrosis.[2]

GROWTH FACTORS INVOLVED IN HEALING

There is a large number of known polypeptide growth factors, some of which act on many cell types and others have restricted cellular targets.

Epidermal Growth Factor (EGF) and Transforming Growth Factor- α (TGF- α)

These two factors belong to the EGF family and share a common receptor. EGF was discovered by its ability to cause precocious tooth eruption and eyelid opening in newborn mice. EGF is mitogenic for a variety of epithelial cells, hepatocytes and fibroblasts. It is widely distributed in tissue secretions and fluids, such as sweat, saliva, urine and intestinal contents. In healing wounds of the skin, EGF is produced by keratinocytes, macrophages and other inflammatory cells that migrate into the area. EGF binds to a receptor (EGFR) with intrinsic tyrosine kinase activity and triggering the signal transduction events. TGF-α has homology with EGF, binds to EGFR and produces most of the biologic activities of EGF.[3]

Hepatocyte Growth Factor (HGF)

HGF was originally isolated from platelets and serum. It has mitogenic effects in most epithelial cells, including hepatocytes and cells of the biliary epithelium in the liver and epithelial cells of the lungs, mammary gland, skin and other tissues. Besides its mitogenic effects, HGF acts as a morphogen in embryonic development and promotes cell scattering and migration. This factor is produced by fibroblasts, endothelial cells and liver nonparenchymal cells. The receptor for HGF is the product of the proto-oncogene cMET, which is frequently over expressed in human tumors. HGF signaling is required for survival during embryonic development.[4]

Vascular Endothelial Growth Factor (VEGF)

VEGF is a family of peptides that includes VEGF-A (referred throughout as VEGF), VEGF-B, VEGF-C, VEGF-D and placental growth factor. VEGF is a potent inducer of blood vessel formation in early development *(vasculogenesis)* and has a central role in the growth of new

blood vessels *(angiogenesis)* in adults. It promotes angiogenesis in tumors, chronic inflammation and healing of wounds. VEGF family members signal through three tyrosine kinase receptors: VEGFR-l, VEGFR-2 and VEGFR-3. VEGFR-2 is located in endothelial cells and is the main receptor for the vasculogenic and angiogenic effects of VEGF.[5]

Platelet-Derived Growth Factor (PDGF)

PDGF is a family of several closely related proteins, each consisting of two chains designated A and B. All three isoforms of PDGF (AA, AB and BB) are secreted and are biologically active. PDGF isoforms exert their effects by binding to two cell-surface receptors, designated PDGFR α and β, which have different ligand specificities. PDGF causes migration and proliferation of fibroblasts, smooth muscle cells and monocytes, as demonstrated by defects in these functions in mice deficient in either the A or the B chain of PDGF.[6,7]

Fibroblast Growth Factor (FGF)

This is a family of growth factors containing more than 10 members, of which acidic FGF (aFGF, or FGF-l) and basic FGF (bFGF, or FGF2) are the best characterized. FGF-l and FGF-2 are made by a variety of cells. Released FGFs associate with heparan sulfate in the ECM, which can serve as a reservoir for storing inactive factors. A large number of functions are attributed to FGFs are - new blood vessel formation (angiogenesis), wound repair, development and hematopoiesis.[7]

TGF-β and Related Growth Factors

TGF-β belongs to a family of homologous polypeptides that includes three TGF-β isoforms (TGF-β_l, TGF-β_2, TGF-β_3) and factors with wide ranging functions, such as bone morphogenetic proteins (BMPs), activins, inhibins and mullerian inhibiting substance.[3]

It is a homodimeric protein produced by a variety of different cell types, including platelets, endothelial cells, lymphocytes and macrophages. Active TGF-β binds to two cell surface receptors (types I and II) with serine/threonine kinase activity and triggers the phosphorylation of cytoplasmic transcription factors called *Smads.* TGF-β first binds to a type II receptor, which then forms a complex with a type I receptor, leading to the phosphorylation of Smad 2 and 3. Phosphorylated Smad2 and 3 form heterodimers with Smad4, which enter the nucleus and associate with other DNA-binding proteins to activate or inhibit gene transcription. TGF-β has multiple and often opposing effects depending on the tissue and the type of injury. TGF-β is a growth inhibitor for most epithelial cell types and for

leukocytes.[2] TGF-β is a potent fibrogenic agent that stimulates fibroblast chemotaxis, enhances the production of collagen, fibronectin and proteoglycans. It inhibits collagen degradation by decreasing matrix proteases and increasing protease inhibitor activities. TGF-β has a strong anti-inflammatory effect.[3]

SIGNALING MECHANISMS IN CELL GROWTH

Cell proliferation is a tightly regulated process that involves a large number of molecules and interrelated pathways. The first event that initiates cell proliferation is, usually, the binding of a signaling molecule, the *ligand*, to a specific cell *receptor*.

Based on the source of the ligand and the location of its receptors-in the same, adjacent, or distant cells-three general modes of signaling, named *autocrine*, *paracrine* and *endocrine*, can be distinguished.

Autocrine signaling

Cells respond to the signaling molecules that they themselves secrete, thus establishing an autocrine loop. Several polypeptide growth factors and cytokines act in this manner.

Paracrine signaling

One cell type produces the ligand, which then acts on adjacent target cells that express the appropriate receptors. The responding cells are in close proximity to the ligand-producing cell and are generally of a different type.

Endocrine signaling

Hormones are synthesized by cells of endocrine organs and act on target cells distant from their site of synthesis, being usually carried by the blood. E.g. -Growth factors like HGF and cytokines.[8]

RECEPTORS AND SIGNAL TRANSDUCTION PATHWAYS

The binding of a ligand to its receptor triggers a series of events by which extracellular signals are transduced into the cell and modulate changes in gene expression. Receptors are generally located on the surface of the target cell but can also be found in the cytoplasm or nucleus. A receptor protein has binding specificity for particular ligands and the resulting receptor-ligand complex may initiate specific or multiple cellular responses. There are various types of receptors.[2]

Receptors with intrinsic tyrosine kinase activity

The ligands for receptors with tyrosine kinase activity include most growth factors such as EGF (epidermal growth factor), TGF-α (transforming growth factor- α), HGF (hepatocyte growth factor/scatter factor), PDGF (platelet- I derived growth factor), VEGF (vascular endothelial growth factor) and FGF (fibroblast growth factor). Receptors belonging to this family have an extra cellular ligand-binding domain, a transmembrane region and a cytoplasmic tail that has intrinsic tyrosine kinase activity. Binding of the ligand induces *dimerization of the receptor,* tyrosine phosphorylation and activation of the receptor tyrosine kinase. The active kinase then phosphorylates and thereby activates, many downstream *effector molecules* (molecules that mediate the effects of receptor engagement with a ligand). Phosphorylated residues in the receptor also serve as docking sites for adapter molecules that bind effector molecules. Effector molecules include *phospholipase* Cγ(PLCγ) and PI-3 kinase. PLCγ catalyzes the breakdown of membrane inositol phospholipids into two products-inositol triphosphate (IP_3), which functions to increase concentrations of another important effector molecule, calcium; and diacylglycerol, which activates the serine-threonine kinase protein kinase C (PKC), which in turn activates various transcription factors. PI_3 kinase phosphorylates a membrane phospholipid, generating products that activate the kinase Akt (also referred to as protein kinase B). Akt is involved in cell proliferation and in inhibition of apoptosis.[9]

Receptors lacking intrinsic tyrosine kinase activity that recruit kinases

Ligands for these receptors include many cytokines, such as interleukin-2 (IL-2), lL-3 and other interleukins; interferons α, β, and γ; erythropoietin; granulocyte colony-stimulating factor; growth hormone; and prolactin. These receptors transmit extracellular signals to the nucleus by activating members of the JAK (Janus kinase) family of proteins. The JAKs link the receptors with and activate cytoplasmic transcription factors called STATs (signal transducers and activation of transcription), which directly shuttle into the nucleus and activate gene transcription.[9]

Transmembrane G-protein-coupled receptors (GPCRs)

These receptors were so named because they contain seven transmembrane α-helices. They constitute the largest family of plasma membrane receptors and transmit signals into the cell through trimeric GTP-binding proteins *(G-proteins).* Binding of the ligand induces changes in the conformation of the receptors, causing their activation and allowing their interaction with

many different G-proteins. Activation of G-proteins occurs by the exchange of GDP, present in the inactive protein, with GTP, in the active protein. Among the many branches of this signal transduction pathway are those involving calcium and adenosine 3', 5'-cyclic monophosphate (cAMP) as second messengers. Activation of seven transmembrane G-protein-coupled receptors can produce inositol 1,4, 5 triphosphate (IP3), which releases calcium from the endoplasmic reticulum. Calcium signals, which are generally oscillatory, have a multiplicity of targets, including cytoskeletal proteins, chloride- and potassium-activated ion pumps, enzymes such as calpain and calcium-binding proteins such as calmodulin.[9]

Steroid hormone receptors

The ligands for these receptors diffuse through the cell membrane and bind to receptors located in the nucleus or less frequently in the cytoplasm. They are involved in a broad range of responses that include cell differentiation and adipogenesis.[3]

Extracellular Matrix (Ecm) and Cell-Matrix Interactions

The ECM is secreted locally and assembles into a network in the spaces surrounding cells. It forms a significant proportion of the volume of any tissue. The ECM serves many functions. For example, matrix proteins sequester water that provides turgor to soft tissues and minerals that give rigidity to skeletal tissues. They also function as a reservoir for growth factors controlling cell proliferation. ECM is important for cell-to-cell interactions and provides a substratum for cells to adhere, migrate, and proliferate, directly modulating cell form and function. Synthesis and degradation of ECM accompanies morphogenesis, wound healing, and chronic fibrotic processes, as well as tumor invasion and metastasis.

Three groups of macromolecules, which are often physically associated, constitute the ECM: (1) *fibrous structural proteins,* such as the collagens and elastins; (2) a diverse group of *adhesive glycoproteins;* and (3) *proteoglycans and hyaluronic acid.* These macromolecules are present in intercellular junctions and cell surfaces and may assemble into two general organizations: *interstitial matrix* and *basement membrane (BM).* The interstitial matrix is present in spaces between epithelial, endothelial and smooth muscle cells and in connective tissue. BMs are produced by epithelial and mesenchymal cells and are closely associated with the cell surface. The integrins bind ECM components and interact with cytoskeleton at focal adhesion complexes. This can initiate the production of intracellular messengers or can directly mediate nuclear signals. Cell surface receptors for growth factors may activate signal

transduction pathways that overlap with those activated by integrins. Collectively, these are integrated by the cell to yield various responses, including changes in cell growth and differentiation.[10]

Growth Factors and Receptors Involved in Angiogenesis

Many growth factors exhibit angiogenic activity, but most evidence points to a special role for *VEGF* and the *angiopoietins* in embryonic vasculogenesis and adult angiogenesis. VEGF is secreted by many mesenchymal and stromal cells, but VEGFR-2, a tyrosine kinase receptor that is the most important in angiogenesis, is largely restricted to endothelial cells and their precursors. In angiogenesis involving endothelial cell precursors, VEGF, acting through VEGFR-2, stimulates the mobilization of endothelial cell precursors from the bone marrow and enhances the proliferation and differentiation of these cells at the site of angiogenesis. Endothelial cell proliferation, differentiation, and migration can also be enhanced by FGF-2.[11]

Newly formed vessels are fragile and need to become "stabilized." Stabilization requires the recruitment of pericytes and smooth muscle cells and the deposition of ECM proteins. *Angiopoietins land* 2 *(Ang1 and Ang2),* PDGF, and TGF-β participate in the stabilization process.[12]

Fibroblast Migration and Proliferation

Granulation tissue contains numerous newly formed blood vessels. As discussed previously, VEGF promotes angiogenesis but is also responsible for a marked increase in vascular permeability (VEGF was first named vascular permeability factor). The latter activity leads to exudation and deposition of plasma proteins, such as fibrinogen and plasma fibronectin, in the ECM and provides a provisional stroma for fibroblast and endothelial cell ingrowth. *Migration* of fibroblasts to the site of injury and their subsequent *proliferation* are triggered by multiple growth factors, including TGF-β, PDGF, EGF, FGF and the cytokines IL-l and TNF.[2]

The sources of these growth factors and cytokines include platelets, a variety of inflammatory cells (notably macrophages) and activated endothelium. Macrophages are important cellular constituents of granulation tissue, clearing extracellular debris, fibrin and other foreign material at the site of repair. Of the growth factors involved in inflammatory fibrosis, TGF-β appears to be the most important because of the multitude of effects that favor fibrous tissue

deposition.[1] TGF-β is produced by most of the cells in granulation tissue and causes fibroblast migration and proliferation, increased synthesis of collagen and fibronectin and decreased degradation of ECM by metalloproteinases. TGF-β is also chemotactic for monocytes and causes angiogenesis in vivo, possibly by inducing macrophage influx.[3]

ECM Deposition and Scar Formation

As repair continues, the number of proliferating endothelial cells and fibroblasts decreases. Fibroblasts progressively deposit increased amounts of ECM. Fibrillar collagens form a major portion of the connective tissue in repair sites and are important for the development of strength in healing wounds. Collagen synthesis by fibroblasts begins within 3 to 5 days after injury and continues for several weeks, depending on the size of wound. Many of the same growth factors that regulate fibroblast proliferation also stimulate ECM synthesis.[13] Ultimately, the granulation tissue scaffolding is converted into a scar composed of spindle-shaped fibroblasts, dense collagen, fragments of elastic tissue and other ECM components. As the scar matures, vascular regression continues, eventually transforming the richly vascularized granulation tissue into a pale, avascular scar.[2]

Tissue Remodeling

The replacement of granulation tissue with a scar involves transitions in the composition of the ECM. Some of the growth factors that stimulate synthesis of collagen and other connective tissue molecules also modulate the synthesis and activation of metalloproteinases, enzymes that degrade these ECM components. The balance between ECM synthesis and degradation results in *remodeling* of the connective tissue framework-an important feature of both chronic inflammation and wound repair. Degradation of collagen and other ECM proteins is achieved by a family of matrix metalloproteinases (MMPs), which are dependent on zinc ions for their activity. This family of enzymes, which includes more than 20 members, has in common a 180 residue zinc-protease domain.[8]

CONCLUSION

This review provides the information regarding the role of growth factors and signalling mechanisms in wound healing. Understanding the signalling events that regulate wound healing in order to completely regenerate damaged tissue may suggest advanced treatment modalities for improving wound healing outcomes in future. To achieve these goals, further research is needed to elucidate the mechanism of ectomesenchymal interaction involved in wound healing.

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