Platelet-Derived Factors Involved in Tissue Repair—From Signal to Function

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Topical treatment with platelet derivatives has increasingly been described as being capable of accelerating wound healing and to aid in tissue repair. In vitro data indicate that platelets and their contents have chemotactic, migration-inducing, and mitogenic activities, and a major role of these factors in tissue repair has thus been advocated. However, how platelet-derived factors orchestrate tissue repair at the cellular level remains quite obscure even to those individuals who prescribe platelet derivatives as topical wound healing therapy. The primary objective of this review was to provide the practitioner, inexpert in biochemistry, an overview about signal transduction within cells in response to platelet-derived factors. Concepts from the literature were selected to illustrate how a relatively few units of information can be put together in specific order to allow for complex biologic functions to be elicited. To illustrate how functional complexity emerges from a narrow set of messengers, an analogy between signal transduction and language, or contrapunctual music, has been drawn.

SEVERAL STUDIES HAVE been published that support the use of a variety of platelet-derived components to improve healing and tissue regeneration achieved by delivering stored platelet-derived growth factors (PDGFs) to damaged tissue.[1-3] There is an increasing understanding about the mechanisms through which these PDGFs might accelerate tissue repair.[4,5] Unfortunately, neither clinical outcomes nor biochemical mechanisms are sustained by a comprehensive literature on the topic. The relative absence of randomized clinical trials is due to a combination of at least 2 sources of product variability: (i) differences in biologic preparations used aiming to achieve healing[6] and (ii) individual variability of patients and the types of lesions to be treated. Physicians who use platelet-derived blood components such as topical multiple growth factor therapy know little about the biochemistry of these growth factors or their functions. This is primarily due to the complexity of the biochemical pathways involved and their intricate description in the literature. This review aimed to provide physicians with the fundamentals of platelets and their growth factors, with specific reference to their role in wound healing.

In his Nobel lecture on December 8, 1984, NK Jerne drew "an analogy between linguistics and immunology."[7] Analogously, we believe that PDGFs can induce signal transduction that may be similar to specialized language, made of specific words, phrases, and grammar. Similar to spoken languages, some words have unambiguous interpretation, whereas others can have diverse connotations (ie, mutatis mutandis, diverse biologic function) depending on the context in what they are used. Platelets are associated with dozens of bioactive components that can be released under various physiologic and pathologic conditions. In selected clinical conditions such as chronic ulcers, acute wounds, bone nonunion, dental implants, and others potential applications of platelet-derived components that aim to enhance healing by providing damaged tissues with PDGFs. The most studied of these factors are PDGF, fibroblast growth factor (FGF), transforming growth factor β (TGFβ), epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), and insulin-like growth factor (IGF). A complete list of abbreviations used in this article is provided in Table 1. Embryogenesis, tissue development, and wound or tissue repair use nearly identical biochemical machinery. However, each one of these conditions also involves specific exclusive biochemical pathways that are characterized by typical induction mechanisms and control systems. Cellular and humoral mechanisms involved in both development and repair are highly conserved through evolution.[8] Healing of an acute wound occurs through 3

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Table 1. Abbreviations Used in This Article

Platelet growth factors and their receptors	
bFGF or FGF2	Basic fibroblast growth factor
CTGF	Connective tissue growth factor
EGF	Epidermal growth factor
EGFR	Epidermal growth factor receptor
FGFR	Fibroblast growth factor receptor
GPCR	G protein-coupled receptor
HB-EGF	Heparin bound epidermal growth factor
IGF-1	Insulin-like growth factor-1
IGF1R	Insulin-like growth factor-1 receptor
PDGF or PDGFs	Platelet-derived growth factor(s)
PDGF-A, -B, -AB	Platelet-derived growth factor isomers A, B, AB
PDGFR	Platelet-derived growth factor receptor
PDGFRα	Platelet-derived growth factor receptor α
PDGFRβ	Platelet-derived growth factor receptor β
TGFβ	Transforming growth factor β
TGFβRI	Type 1 transforming growth factor β receptor
TGFβRII	Type 2 transforming growth factor β receptor
VEGF	Vascular endothelial growth factor
VEGFR1	Vascular endothelial growth factor receptor 1
VEGFR2	Vascular endothelial growth factor receptor 2
Other factors relevant to signal transduction	
ECM	Extracellular matrix
eNOS	Endothelial nitric oxide synthetase
ERK	Extracellular signal-regulated kinase
IP3	Inositol trisphosphate
JNK	c-Jun N-terminal kinase
LAP	Latency-associated peptide
MAPK	Mitogen-activated protein kinases
MEK	MAPK kinase
MMP	Matrix metalloprotease
P13K	Phosphoinositide-3 kinase
PKC	Protein kinase C
PLCγ	Phospholipase C-gamma
ROS	Reactive oxygen species
SMAD	Small mother against decapentaplegic
uPA	Urokinase-type plasminogen activator
VRAP	VEGF receptor-associated protein

sequential and partially overlapping phases: inflammation, proliferation, and remodeling. The following is a simplified outline of the cutaneous wound healing process. A blood clot forms immediately after wounding, and this process involves blood cells including platelets, fibrin, and extracellular matrix (ECM) proteins such as fibronectin, vitronectin, and thrombospondin. The clot acts as a physical barrier against microbial infections, is a reservoir of growth factors and cytokines, and provides a favorable environment for cell migration. Neutrophils appear within a few minutes, followed by monocytes and lymphocytes. The proliferation phase then starts with the migration and proliferation of keratinocytes followed by the proliferation of fibroblasts. Angiogenesis and nerve sprouting occur later. After a few days, transition from granulation tissue to scarring and remodeling occurs. The entire process is regulated by various growth factors and cytokines.[9]

Several clinical coincidents such as edema, ischemia, infection, excessive inflammation, metabolic derangements, local pressure, impairment or distorted cytokine levels, or aberrant protease networks may delay tissue repair leading to progression to ulcer formation and to chronicity, thus, making the management of such lesions quite difficult. In such cases, a comprehensive management protocol including local growth factor administration through platelet derivatives seems to be clinically effective.[10]

Wound healing is a complex biologic process that includes the active participation of cells, connective tissue, ECM, and soluble factors, with continuous crosstalk among these elements. The ECM provides structural and functional support to the cells and tissues, and it consists of several molecules such as collagen, proteoglycans, heparan sulfate, chondroitin sulfate, hyaluronic acid, elastin, fibronectin, and laminin. A few plasma-derived proteins such as fibrin, thrombospondin, and fibronectin act as provisional ECM. Soluble factors include cytokines, chemokines, hormones, nucleotides, electrolytes, free ions, and growth factors. In this review, we will focus primarily on the platelet-derived growth factors PDGF, EGF, VEGF, TGFβ, basic FGF (bFGF), and IGF-1, which are known to have vital role during wound/tissue repair and regeneration. We examine these growth factors and their receptors and the ways they transduce their signals through the cytoplasm to the nucleus to

modulate gene expression necessary for specific cellular function to be elicited.

PLATELET-DERIVED GROWTH FACTORS

Platelet-derived growth factors are small or relatively small polypeptides. Most of their receptors belong to the family of the protein tyrosine kinases. The major characteristics of the PDGFs discussed in this review are summarized in Table 2. Platelet-derived growth factors consist of 2 related peptide chains, α (16-kDa, 124 amino acids), and β (14-kDa, 140 amino acids) linked by disulfide bonds. There are 3 active isoforms, αα, ββ, and αβ, as well as 2 kinds of receptors (PDGFRα and PDGFRβ). The PDGFs are highly conserved through evolution: Xenopus and human polypeptides share 40% to 50% consensus sequences. Vascular EGF is a homodimeric protein of 46 to 48 kDa (24-kDa subunits). Bovine and human sequences show 95% homology.

Endothelial growth factor is a globular protein of 6.4 kDa consisting of 53 amino acids. The EGF proteins are evolutionary closely conserved; homology between human EGF and EGF isolated from other species is roughly 70%. Endothelial growth factor is synthesized as a 1207-amino acids long precursor from which the short biologically active peptide is released by proteolytic cleavage.

Transforming growth factor β is a family of disulfide-linked homodimers and heterodimers. The isoforms of TGFβ arise by proteolytic cleavage of longer precursors (12.5-kDa monomeric subunits, 112 amino acids, derived from 304-412 amino acids peptides). Isoforms isolated from different species have sequence identities on the order of 98%.

Basic FGF is an 18-kDa protein with 155 amino acids. The sequences of bovine and human bFGF differ in only 2 amino acids. Human and ovine bFGF are identical. A homolog of human bFGF isolated from *Xenopus laevis* shows an overall sequence homology of 84%.

Insulin-like growth factor-1 is a 7.6-kDa protein with 70 amino acids having 47% identity with insulin. Insulin-like growth factor-1 is highly conserved through vertebrate and invertebrate evolution.

MECHANISMS OF SIGNAL TRANSDUCTION

Extracellular signals are transformed to specific functions within the cell through a cascade of events generally known as signal transduction. The interaction of a cell membrane receptor with its ligand induces a conformational change in the receptor. Consequently, it leads to phosphorylation of the intracellular receptor domain, which in turn triggers a cascade of events where several

Table 2. Major Characteristics of Platelet-Derived Factors

	Growth factor protein	Receptor type	Action under physiologic conditions	Major functions	References
PDGF	30-kDa (α16-kDa; β 14-kDa)	Tyrosine kinase	Autocrine & paracrine	Mitogen; chemotactic	[11,12]
VEGF	46-48 kDa (two 24-kDa subunits)	Tyrosine kinase	Autocrine & paracrine	Mitogen for vascular endothelial cells; angiogenic; chemoattractant for monocytes; induces the synthesis of the metalloproteinase, interstitial collagenase; differentiation of adipocytes	[13-15]
EGF	6.4-kDa	Tyrosine kinase	Autocrine	Mitogen; proliferation, differentiation, angiogenesis	[15-17]
TGFβ	12.5-kDa	Serine/threonine kinase	Autocrine & paracrine	Growth inhibitor for normal and transformed epithelial cells, endothelial cells, fibroblasts, neuronal cells, lymphoid cells; angiogenesis; stimulates the growth of some cell types of mesenchymal origin including fibroblasts and osteoblasts; induces the synthesis of bone matrix and ECM	[15,18,19]
bFGF	18-kDa	Tyrosine kinase	Autocrine & paracrine	Mitogen; differentiation; angiogenesis	[9,15,20]
IGF1	7.6-kDa	Tyrosine kinase	Autocrine & paracrine	Mitogen, differentiation	[21,22]

NOTE. General information from COPE (Cytokines & Cells Online Pathfinder Encyclopedia): www.copewithcytokines.de/cope.cgi.

messenger molecules are phosphorylated. The signal thus passes from the cytoplasm into the nucleus where DNA-binding proteins bind to regulatory DNA sequences leading to DNA replication or transcription. The DNA-mediated response is returned to the cytoplasm via messenger RNA that is translated into functional protein, which in turn modulates cellular functions attributed to the initial signal. One of the initial events in the signaling cascade is known to be the activation of tyrosine kinase, which phosphorylates the tyrosine residues of the target protein. It has been shown that activated tyrosine kinase leads to phosphorylation of receptors at the tyrosine residue that then leads the receptor to be complexed with several other proteins including phosphoinositide-3 kinase (PI3K), phospholipase C-γ (PLCγ), GTPase-activating protein, and raf1. Downstream activation includes several mediators, for example, mitogen-activated protein kinases (MAPK), PI3K, PLCγ; several tyrosine kinases, as well as others.[11,12] In some situations, serine/threonine kinase is activated that in turn phosphorylates the serine/threonine residues of the target protein. In these cases, the receptor phosphorylation activates the kinase domain, which in turn activates the transcription factors known as Smads (small mother against decapentaplegic). Each Smad can interact with several DNA-binding proteins leading to diverse gene transcripts, thus, generating distinct biologic response.[23]

Generation of Specificity—How Signals Are Converted in Specific Functions

Considerable information is available regarding the general mechanisms of receptor-mediated downstream signaling pathways. However, the generation of the specificity of biologic response remains quite obscure. There are several hypotheses, each one based on experimental evidence, that indicate that (*a*) receptors switch a generic on-off signal, (*b*) receptors generate specific cell response activating specific pathway basing on the strength of the signal,[11] and (*c*) receptors generate specific cell responses activating a specific pathway depending on the duration of activation.[24,25] Of course, in vitro analyses are restricted to simple signaling events and may not reflect the complex signaling cascade that probably occurs in vivo. It is very likely that the specificity of the biologic response achieved in vivo relies on both qualitative and quantitative differences in the stimulation perceived by a particular receptor, which is dependent on either ligand concentration or permanence, receptor expression, and also on the immediate microenvironment.[26,27] For example, it has been demonstrated that a low concentration of PDGF induces PDGFRα activation, which triggers the PI3K pathway leading to gene transduction having to do with cell migration, whereas a high ligand concentration triggers PLCγ pathway and cell proliferation.[25] Furthermore, ECM proteins, their receptors, and protease activities have crucial roles in determining the perception of the microenvironment context from the responding cell leading to a context-dependent cell response. It is likely that these factors induce phosphorylation of specific proteins thus eliciting distinct biologic responses through various downstream cascades.[26-28] In contrast to the perceived simplicity of the signal transduction mechanism via a limited number of proteins, specificity of cellular responses to growth factor ligand-receptor binding is very complex being simultaneously influenced by several regulatory mechanisms including cross talking with other pathways and context dependency.[29,30]

In a multicellular organism, each cell has adopted a relatively limited number of signal transducer proteins thereby adapting them to their exact specificity. This architecture is similar to that of thousand languages spoken by humans all over the world using a limited set of letters or that of each single language where meaning of words and sentences is driven by context, environment, and person-to-person relationships.

Downstream Signal Transduction

Platelet-derived growth factor. The PDGF genes and their corresponding polypeptides belong to a family of structurally and functionally related growth factors including VEGFs. It is very likely that both ligands and receptors derived from an ancestral VEGF system through duplication and divergent evolution.[11] High conservation through evolution of PDGF/VEGF and their related receptors PDGFR/VEGFR is testifying conservation among species of common mechanisms and effectors required for fundamental survival of the organism such as development and the healing of lesions.[13]

Signal transduction of PDGF and VEGF stimulation occurs through structurally related receptor

tyrosine kinases (RTKs)[12] (Fig 1). Several downstream gene transductions regulating actin reorganization, cell movement, and inhibition of apoptosis are induced by PI3K activation. Phosphorylation of PLCγ includes direct activation of protein kinase C and regulation of calcium ions influx. A heterogeneous group of downstream kinase mediators is involved in several and diverse cell functions including chemotaxis, cell migration, inflammation, differentiation, and cell survival. They can be subdivided in 3 major families: extracellular signal-regulated kinase (ERK), p38 kinase, and c-Jun N-terminal kinase.[31]

In addition to the common signal transduction pathway illustrated in Figure 1, several other factors could also influence the signaling cascade. For example, interactions between transcription factors, cross talk among receptors and downstream activation signals are reported in certain instances such as PDGFR activation. For example, after ligand binding to PDGFR, it leads to induction or repression of "immediate early genes" (IEGs). Immediate early genes are a large set of conserved genes quickly activated by several receptors; they support several, diverse, and even contrasting cell functions.[32] Functions triggered via IEGs seem to be induced quantitatively rather than qualitatively. Cells seem to respond to IEGs, which is analogous to loudly shouting sergeant orders instead of gentle captain speaking.

The PDGF receptors interact with other cell membrane-associated components (eg, integrins) and cross talk with other receptors. Interaction with integrins increases cell survival and enhances

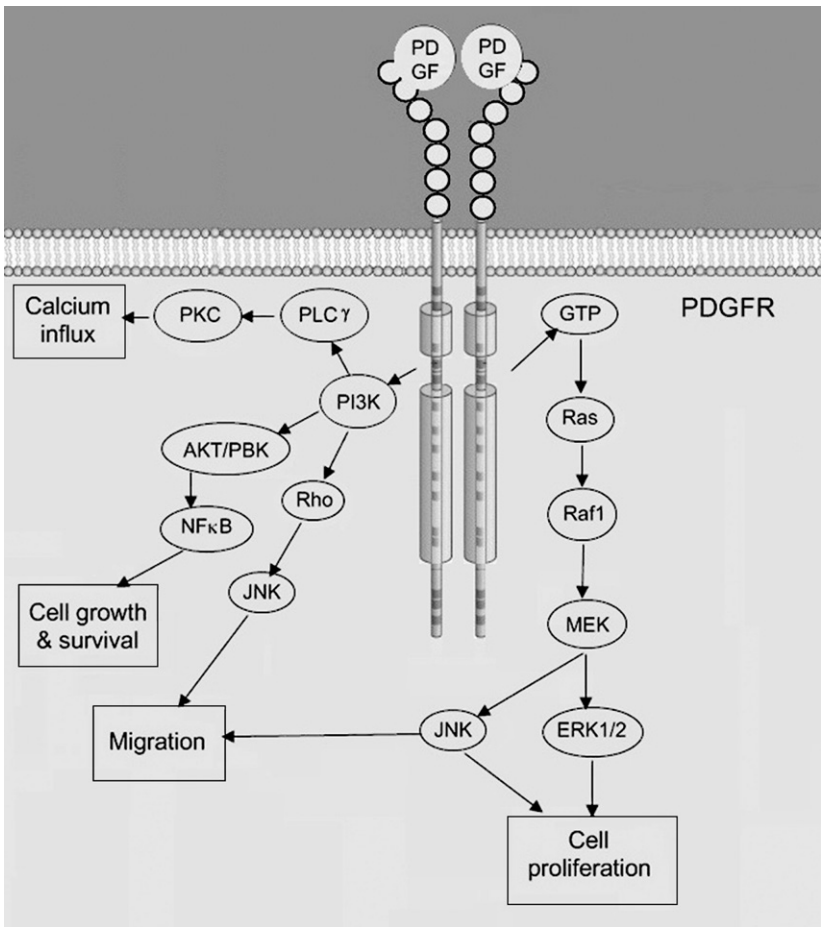

Fig 1. The PDGF signaling through PDGFR. Activation of PI3K is the hinge element for several downstream gene transductions to occur, regulating actin reorganization, cell movement, and inhibition of apoptosis. Calcium ion influx is regulated by sequential activation of PI3K, PLCγ, and PKC. A heterogeneous group of downstream kinase mediators such as ERK, MEK (MAPK kinase), and c-Jun N-terminal kinase is involved in chemotaxis, cell migration, and proliferation.

cell migration and proliferation. Furthermore, it leads to redistribution/localization of PDGFR facilitating cross talk between PDGFR and other receptors.[11] Cross talk with ECM component and receptors is a common feature shared by several growth factor receptors such as PDGFR, TGFβRI, FGFR, EGFR, and VEGFR that regulate several wound repair activities in several cells implicated in this process. For example, fibroblasts combine signals provided by growth factors and matrix. If fibroblasts migrate through a fibrin clot, they up-regulate integrin subunits such as α3 and α5 after exposure to PDGF, whereas in a collagen-rich microenvironment, they up-regulate α2 (collagen-specific) receptor units after exposure to PDGF. Fibroblasts and keratinocytes down-regulate their collagen receptors and up-regulate integrins that bind fibronectin and vitronectin to crawl into the provisional fibrin matrix.[16]

Vascular endothelial growth factor. Vascular endothelial growth factor is a family of PDGFs involved particularly in angiogenesis, neovasculogenesis, and vascular permeabilization. Vascular endothelial growth factor controls wound repair process by inducing vascular permeability, angiogenesis, and recruitment of inflammatory cells and of marrow-derived progenitor cells into the wound site.

In humans, the most important and abundant member is VEGF-A, others being VEGF-B, VEGF-C, VEGF-D. The VEGF system (ligands and receptors) is complex. Besides the VEGR members (A, B, C, D, and platelet growth factor), there are various isoforms ($VEGF_{121}$, $VEGF_{121b}$, $VEGF_{145}$, $VEGF_{165}$, $VEGF_{165b}$, $VEGF_{189}$, $VEGF_{206}$) with different biologic activities and 3 receptors (VEGFR1, VEGFR2, VEGFR3). The VEGF receptors are RTKs. The VEGF signaling is largely mediated by VEGFR2. For simplicity, here we will refer to this factor as VEGF.

An important biologic property that distinguishes the function of different VEGF isoforms is their heparin and heparan sulfate-binding ability testifying the importance of ECM-cell interaction in determining the growth factor-induced biologic functions. The heparin-binding domain is crucial for the binding of VEGF to the ECM molecules. The angiogenic potential of matrix-bound VEGF is superior to that of soluble VEGF. Proteolysis of ECM-associated VEGF isoforms modulates several and diverse functions exerted by VEGF as described here. Vascular EGF_{189} not only binds VEGFR1 but also binds to VEGFR2 after proteolysis, thus, exerting mitogenic effect on endothelial cells. Vascular EGF_{165} is mainly sensitive to serine proteases, plasmin in particular, which reduces VEGF receptor affinity and ECM interaction. In nonhealing wounds, there is an increased plasmin-dependent VEGF proteolysis, which results in presence of decreased levels of $VEGF_{165}$ in these wounds. Besides proteolysis, the soluble VEGFR1 receptor is the only naturally occurring inhibitor for VEGF. It acts as a competitor of cell-associated VEGF receptors. Interestingly, the fluids from the nonhealing wounds have higher concentration of soluble VEGFR1 as compared to the fluid from healing wounds.[33]

Both VEGF and bFGF bind to heparin and to heparan sulfate. In wounded tissue, fibrin forms the temporary matrix for cellular response and subsequent vascular and tissue repair. Endothelial cell proliferation and angiogenesis depend upon complex cell-growth factor-extracellular environment relationship that include fibrin, heparan sulfate, heparin, growth factors (VEGF, bFGF, PDGF-B), growth factor receptors, $\alpha v\beta 3$ integrins, and regulatory proteases such as plasmin.[33-35]

At least 6 pathways are implicated in VEGFR2 downstream signaling (Fig 2) for the 3 major functions to be elicited: vascular permeability; cell survival and proliferation, cytoskeleton rearrangement, and cell migration. Pathways to accomplish these functions (that converge in one super-function, angiogenesis) are summarized as follows.

Vascular permeability. Four distinct pathways are initiated by phosphorylation of VEGFR2 at 2 tyrosine residues, Tyr 951 and Tyr 1175, which sustain vascular permeability. One of the pathways is initiated by PLCγ that activates the cascade inositol trisphosphate, calcium influx, protein kinase C (PKC) activation, and endothelial nitric oxide synthetase (eNOS). The second is initiated by VEGF receptor-associated protein (VRAP) that activates Src/STAT3 pathway that in turn controls either vascular permeability and/or cell migration. The third is that initiated by the adaptor molecule Shb that activate PI3K-AKT-eNOS cascade. Finally, the fourth pathway includes activation of CDC42 and p38 MAPK.

Cell migration. Vascular EGFR2 is known to elicit cell migration via 4 different pathways. One is initiated through activation of CDC42 and p38

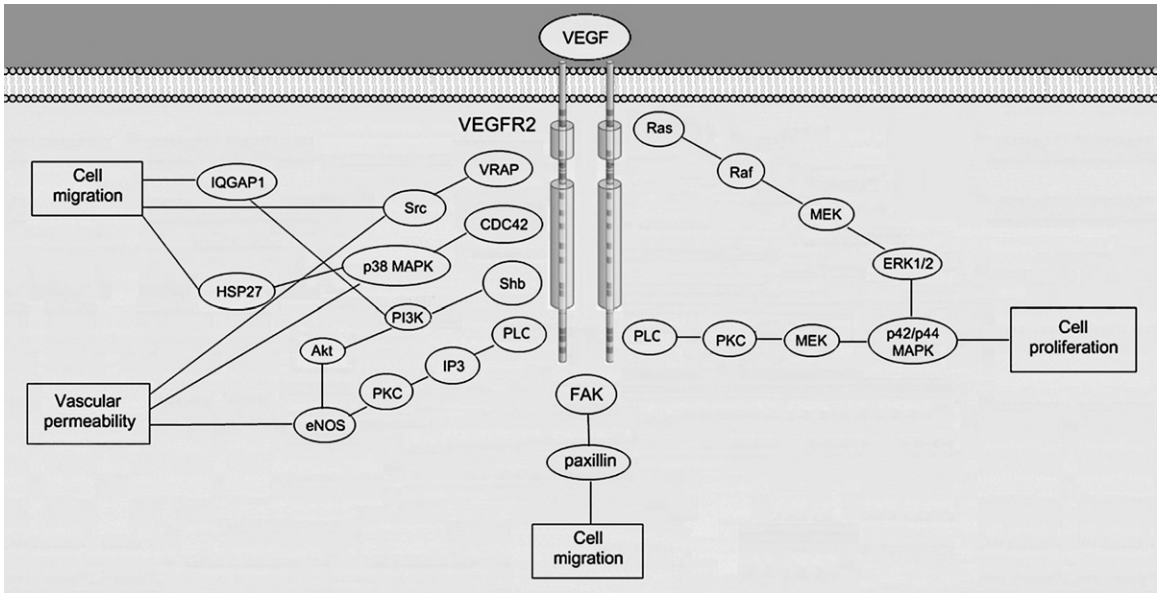

Fig 2. Diagrammatic synthesis of signaling moderated by VEGFR2 leading to diverse cell functions. Several pathways elicit cell functions such as vascular permeability; cytoskeleton rearrangement; and cell migration/proliferation all converging in one super-function, angiogenesis. Vascular permeability is sustained by 4 pathways. One is initiated by PLCγ that activates the cascade inositol trisphosphate (IP3), PKC, and eNOS. One is initiated by VRAP that controls either vascular permeability or cell migration. One is initiated by the adaptor molecule Shb that activate PI3K-AKT-eNOS cascade. One pathway includes activation of CDC42 and p38 MAPK. Four pathways also sustain cell migration. One that is initiated through activation of CDC42 leading to phosphorylation of the heat-shock protein-27 (HSP27) that regulates actin reorganization and cell migration. One that is initiated by Shb-PI3K; then PI3K activates a GTPase-activating protein (IQGAP1) that regulates cell migration. One that is initiated by VRAP as previously described. One includes activation of the focal-adhesion kinase (FAK) and its substrate paxillin, which are involved in cell migration. Cell proliferation is sustained by 2 distinct signaling pathways that have one common intermediate factor, MAPK kinase (MEK1/2). The first and more important pathway is that initiated by PLCγ that leads to the activation of PKC, MEK1/2, and p42/p44 MAPK. The second pathway is the classical Ras-Raf-MEK-MAPK cascade.

MAPK. p38 MAPK induces phosphorylation of the heat-shock protein-27 that regulates actin reorganization and cell migration. A second is that initiated by Shb-PI3K. Phosphoinositide-3 kinase activates a GTPase-activating protein, which positively regulates cell migration. A third is that initiated by VRAP and described above. The fourth pathway includes the focal-adhesion kinase and its substrate paxillin, which are involved in focal-adhesion turnover during cellular migration. Cell migration is also actively modulated by VGFR1 through PI3K phosphorylation and several downstream mediators involved in signal transduction (Fig 3).

Cell proliferation. Vascular EGFR2 elicits 2 distinct signaling pathways leading to cell proliferation, and these pathways have a single common intermediate factor, MEK1/2. The first and more important pathway is that initiated by PLCγ that leads to the activation of PKC, MEK1/2, p42/p44 MAPK. The second pathway is the classical Ras-Raf-MEK-MAPK cascade.

The ECM component and membrane adhesion molecules regulate the functions of VEGF through VEGFR. Heparin, heparan sulfate, and heparan sulfate proteoglycans (Fig 4) amplify signals from $VEGF_{165}$ but not from $VEGFA_{121}$.[27]

Epidermal growth factor. Epidermal growth factor is a small mitogenic protein involved in cell survival, migration, and proliferation. Epidermal growth factor has high homology with TGFα, which is a competitor for EGF receptor site. A family of ligands (EGF, heparin-binding EGF [HB-EGF], TGFα, amphiregulin) binds to a family of ErbB receptors (ErbB1-4) present in most human cell types. Epidermal growth factor binds with high affinity to ErbB1 (EGFR). Upon ligand binding, EGFRs dimerize and autophosphorylate at tyrosine residues, whereas subsequent activation of ERKs occurs via adaptor complexes and the Ras/Raf pathway.[16] Similar to many other RTKs, EGFRs cooperate (cross talk) with other cell membrane receptors, particularly with G protein-coupled

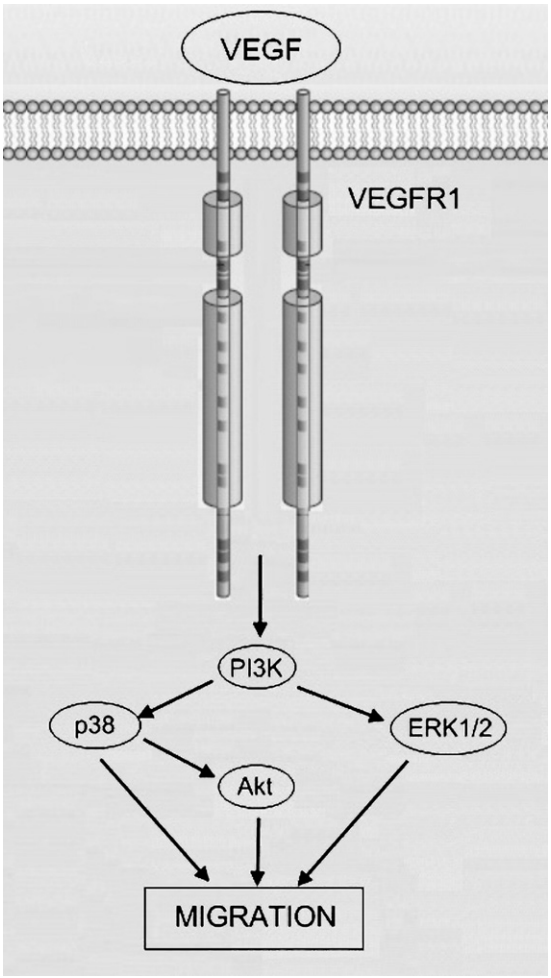

Fig 3. Signaling through VEGFR1 and cell migration. Activation of PI3K is the hinge element for downstream signal transduction leading to cell migration induced through the activation of 3 different kinases: p38, AKT, and ERK1/2.

receptors (GPCRs). G protein-coupled receptors are the larger group of membrane receptors in eukaryotic cells including almost 1000 members. G protein-coupled receptors and RTKs modulate and transactivate each other, thus, cooperating in activating and diffusing their own downstream signaling pathways. Receptor tyrosine kinases, such as VEGFR, EGFR, PDGFR, participates to such transactivation that lead to transcription of genes (p21, p27, cyclins) that induce cell transition from G_1 to S phase during proliferation. Platelet-derived growth factor receptor can be directly transactivated through GPCR activation. To the contrary, EGFR is indirectly transactivated through activation of matrix metalloproteases that in turn dissociate HB-EGF making soluble EGF available to EGFR binding and downstream signaling. Very likely there is a hierarchy in the transactivation of EGFR and PDGFR depending on membrane receptor expression, cross talking with other receptors (eg, integrins), or the specific downstream signaling transducers. Such hierarchy of activation can modulate receptor signaling. For example, in vascular smooth muscle cells, EGFR seem to be more easily transactivated by GPCR, thus, leading to the down-regulation of PDGFR-mediated signaling[36] (Fig 5). The RTK-mediated signaling and GPCR-mediated signaling share several downstream partners including Rac, reactive oxygen species (ROS), PI3K, and MAPK[17,36-38] (Fig 5). Receptors cross talking and transactivation play a very important role in the healing process both in physiologic condition and after topical application of growth factors. Transactivation of EGFR is known to be essential at the initial stage of wound repair process as it is necessary for cell migration.[39] It has been shown that topical application of EGF and PDGF induces phosphorylation of EGFR and PDGFR. Receptor phosphorylation remains for 5 days. Increased and sustained level of EGFR and PDGFR phosphorylation appears to accelerate the wound healing process. Topical application of wounds with EGF and PDGF either individually or in combination results in a coordinated up-regulation of EGFR, PDGFRα, and PDGFRβ proteins. Similarly, the topical application of PDGF-AB and PDGF-B up-regulates EGFR. This up-regulation occurs as early as day 1 after growth factor application, persisting until day 3 and returning to basal level after day 5.[5]

Transforming growth factor β. Transforming growth factor β is a multifunctional peptide that controls proliferation, differentiation, and other functions in many cell types. It also acts as an autocrine-negative cell growth regulator with antiproliferative effect. Many cells synthesize TGFβ and almost all of them have specific receptors for this peptide. The TGFβ isoforms β1, β2, and β3 all function through the same receptor signaling systems. Among these, the isoform TGFβ1 is mostly represented in platelets. Here, we use the general term TGFβ, which would represent all the 3 isoforms. The TGFβ family proteins are produced as precursor proteins. Functional TGFβ is produced by removal of latency-associated peptide from the precursor

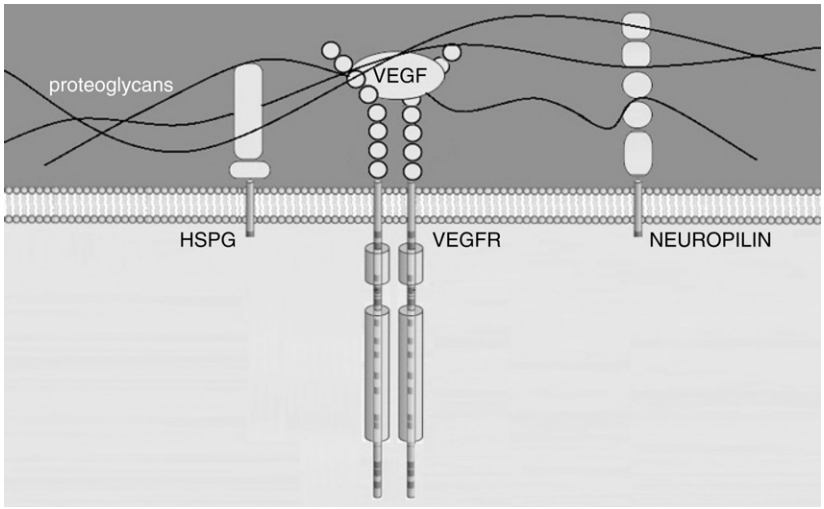

Fig 4. Proteoglycans interplay with VEGFR. Proteoglycans associate with the cell membrane both through heparan sulfate proteoglycan core protein (HSPG), VEGFR, and through the transmembrane coreceptor neuropilin. Tissue tensomechanic forces modulate signals elicited by some VEGF isomers via proteoglycans receptors and coreceptors.

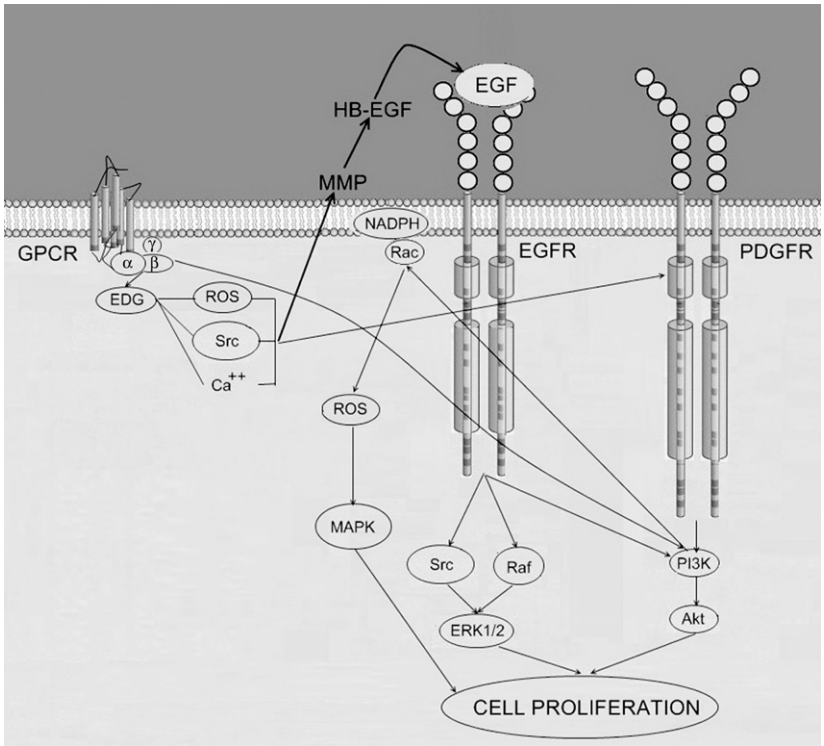

Fig 5. The EGF signaling and receptor transactivation. Upon EGF interaction with its receptor (EGFR), this undergoes dimerization and autophosphorylation. Cell proliferation is induced either through ERK1/2 activation via adaptor complexes and the Ras/Raf pathway or through activation of PI3K and Akt. Endothelial growth factor receptors cross talk with other cell membrane receptors, particularly with GPCRs. This cross talk also involves PDGFRs. After GPCR activation, PDGFR can be directly activated. To the contrary, EGFR is indirectly transactivated through activation of matrix metalloproteases (MMPs) that in turn dissociate HB-EGF making soluble EGF available to EGFR binding and downstream signaling. Signaling exerted by EGFR, PDGFR, and GPCR share several downstream partners including Rac, ROS, PI3K, and MAPK.

protein mostly but not exclusively via proteolytic cleavage by protease and metalloprotease.

The TGFβ signaling system, which includes growth factors, receptors, and Smad-type proteins, has expanded through gene duplication and by adopting new functions through evolution. Conservation and evolution is demonstrated in vertebrate and invertebrates.[18] Signal transduction system of TGFβ super family consists of the following: serine/threonine kinase receptors (in humans 7 type I and 5 type II receptors) and Smad proteins: 2 TGFβ Smads (Smad2, 3), 3 BMP (bone morphogenic protein)-type Smads (Smad1, 5, 8), 1 common mediator co-Smad (Smad4), and 2 inhibitors I-Smads (Smad6, 7).[19]

Upon TGFβ binding, type I and type II receptors (TGFβRI and TGFβRII) associate in tetramers. Type II receptors phosphorylate type I receptors. Such phosphorylation activates the kinase domain that in turn activates Smads. Smads2, 3, and 4 transduce TGFβ signals, whereas Smad6 and 7 modulate/inhibit such signaling cascade (Fig 6). Each Smad can interact with several DNA-binding proteins leading to several, diverse gene transcripts, thus, generating distinct biologic response. The TGFβ family regulates transcription of approximately 300 to 500 genes. The TGFβ downstream signaling pathway maintains active cross talking with other signaling pathways, for example,

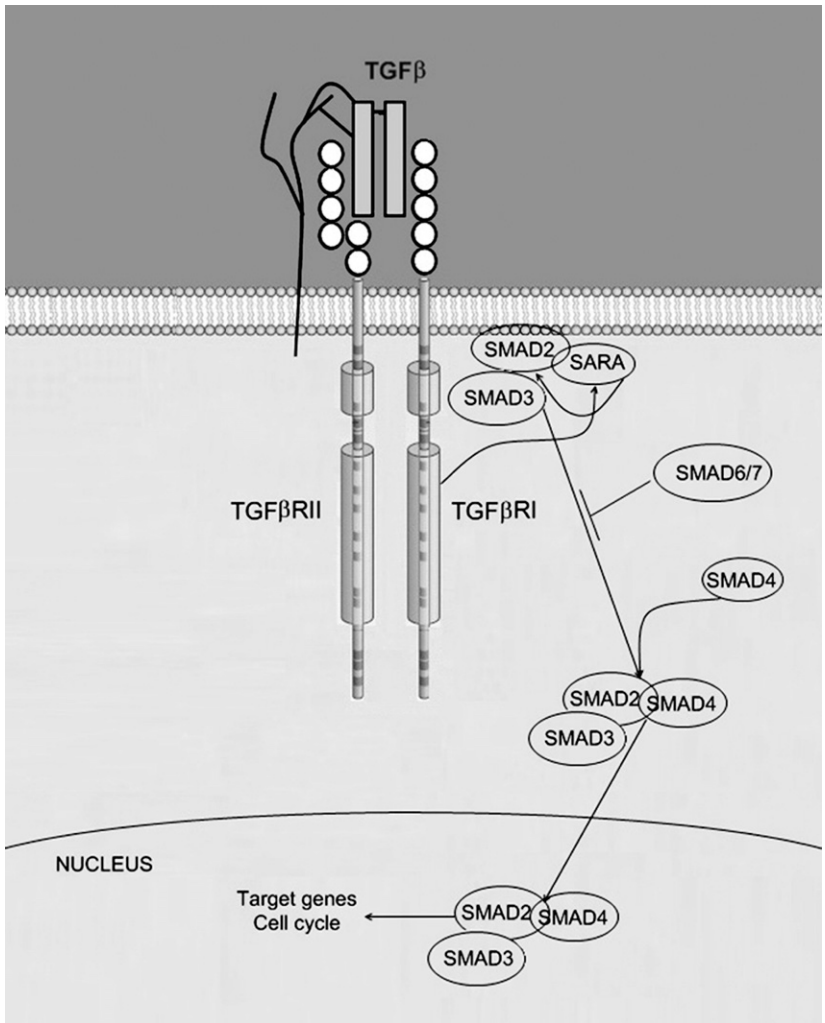

Fig 6. The TGFβ signaling. Upon TGFβ binding, type I and type II receptors (TGFβRI and TGFβRII) associate in tetramers and the type I receptor becomes phosphorylated. The receptor kinase thus activated in turn activates Smads. Smads2, 3, and 4 transduce TGFβ signals, whereas Smad6 and 7 modulate/inhibit such signaling cascade.

receptor tyrosine kinase–activated MAPKs, ρ small GTPase, p38, Akt, and c-Jun N-terminal kinases.[23] Furthermore, TGFβ family signaling also involves non-Smad pathways that regulate the transduction of several sets of genes. Because most of the terminal effectors of TGFβ-generated signals are also recruited by several pathways including those triggered by RTKs and by small GPCRs, the occurrence of cross talk of TGFβ receptors with many other receptors including those modulating the cell-ECM relationships is evident.[30]

At the acute wound site, TGFβ is massively released from platelets, providing an immediate chemotactic signal for inflammatory cells as well as for fibroblasts and keratinocytes. Furthermore, in keratinocytes, TGFβ induces the expression of integrins necessary for cell migration across the fibronectin-rich provisional matrix. Transforming growth factor β is also sequestered within provisional matrix allowing for sustained release of active form for a long time, hence, ensuring continuous provision of TGFβ throughout the entire course of wound repair process. Healing, inflammation, and scarring are strongly dependent upon downstream signal transduction of TGFβ/Smad signaling pathway and that provided by the cognate connective tissue growth factor (CTGF; CCN2), which is also abundantly provided by platelets.[40,41]

Basic FGF. Basic fibroblast growth factor (also known as *FGF-2*) is an 18-kDa protein with 155 amino acids, highly conserved through evolution. Basic FGF is a member of the FGF family, a large group of proteins that have been show to promote cell growth, differentiation, and motility.[20] They transduce their signals through four high-affinity RTKs, FGFR1-4. Common signaling transducers of RTKs elicit various cell functions in accordance with costimulations (Fig 7). Reduced expression or functions of FGF in chronic wounds have been described. High redundancy of FGF family ligand signaling has been demonstrated.[9]

Fibroblast growth factors have high affinity for heparin, heparan sulfate, and proteoglycans, which stabilize tetramer structures formed by 2 molecules of bFGF and 2 molecules of FGFR. This is crucial to FGF signaling from the initial event to the formation of large receptor clusters. During wound healing, the action of heparan sulfate-degrading enzymes activates bFGF, thus, mediating the formation of new blood vessels.[42]

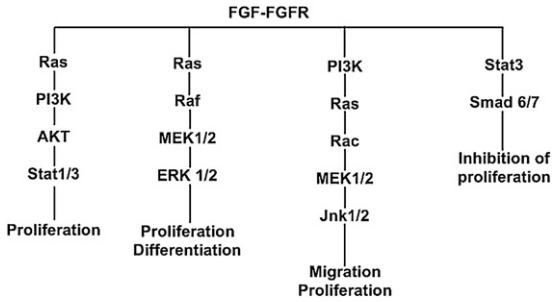

Fig 7. The FGF signal transduction pathways. Fibroblast growth factor has high affinity for heparin. The FGF binding to FGFR is stabilized by heparin. This binding facilitates the formation of tetramers formed by 2 FGF molecules and 2 receptor molecules. Costimulation by HSPG is a driving force for downstream kinase activation. The FGF receptors share most of the kinase repertoire (PI3k, AKT, MEK, ERK, Jnk) with the other RTKs. Activation of the downstream pathway of signal transduction and the elicited functions strongly depend upon costimulation either from tensomechanic forces or from the action of heparan sulfate-degrading enzymes occurring during wound healing and tissue remodeling.

Basic FGF promotes angiogenesis that is exerted synergically with VEGF.[35] This synergism is dependent on complex relationship among growth factors and ECM integrins (in particular $\alpha v\beta 3$, fibrinogen, and fibrin) and the mechanisms of the proteolytic release of VEGF and bFGF from ECM. Endothelial cells up-regulate $\alpha v\beta 3$ integrin expression after exposure to VEGF, and capillary morphogenesis is dependent on proteolysis of surrounding matrix, whereas fibrinogen-bound bFGF requires $\alpha v\beta 3$ binding to up-regulate endothelial cell urokinase-type plasminogen activator.[43] In comparison to soluble growth factors in culture medium, fibrin-bound VEGF and bFGF lead to 6-fold increase of endothelial cell proliferation and angiogenesis.[33] Synergism among growth factors and redundancy of mechanisms of downstream signaling seem to be particularly important to elicit stable results during angiogenesis. Vascular EGF and bFGF produce additive effects, thus, eliciting relatively consistent results. Similarly, quite stable nascent vascular networks are obtained with transient exposure to PDGF-B and bFGF. Combination of growth factors is able to induce transactivation and up-regulation of several receptors (FGFR, VEGFR, PDGFR) allowing for coordinate regulation of vascular growth and remodeling by appropriate agents and effector signals.[5,34]

Insulin-like growth factor-1. Insulin-like growth factor-1 belongs to a hormonal system, which includes IGF polypeptides, IGF receptors, and 6

IGF binding proteins, that vehiculate IGF-1 through the body, and an acid-labile subunit relevant to IGF transport to tissues.[44] It is closely related to the insulin system. In humans, IGF-1 is produced by the liver and to a lesser extent by skeletal muscles, kidney, heart, and lung. Nearly every kind of cell is affected by IGF-1. Within tissues, several cells produce IGF-1 including mesenchymal cells, endothelial and vascular smooth muscle cells, and immune cells. Insulin-like growth factor-1 is a 7.6-kDa protein with 70 amino acids having 47% identity with insulin. Insulin-like growth factor-1 is highly conserved through evolution.[21]

Ligand binding induce autophosphorylation of tyrosine residues of IGF1 receptor (IGF1R), activating multiple downstream signal transduction cascades, thus, inducing the Ras, Raf, MEK, ERK, Elk signaling cascade, and the PI3K-Akt pathway[45] (Fig 8). These pathways are involved in cell growth and inhibition of apoptosis. The PI3K-Akt pathway triggers 2 major downstream effector mechanisms at gene transduction level. One, mediated by activation of the transcription factor nuclear factor (NF)-κB activation, leads to protein synthesis,[46] the other mediated by inhibition of the transcription factor FOXO, leads to cell survival through inhibition of apoptosis.[47,48]

Insulin-like growth factor-1 circulates in the plasma associated to IGF-binding proteins (IGFBPs). Approximately 80% of IGF-1 is vehiculated by IGFBP3.[49] Modulation of local delivery of IGF-1 to tissue is regulated by the acid-labile subunit, which form a stable circulating complex together with a single IGF peptide and a single IGFBP3 molecule.[50] Insulin-like growth factor-1 delivery to tissue is also modulated by heparin-binding IGFBPs. Insulin-like growth factor BP3 and IGFBP5 bind heparin through highly conserved 18-amino acid heparin binding domain: this binding induces change of conformation of IGFBPs resulting in significantly lower affinity for IGF-1. Consequently, the released IGF-1 binds to IGF1R. Insulin-like growth factor BP3 also binds fibrinogen in the fluid phase, thus, modulating IGF-1 binding in fibrin clot and IGF-1 availability to cells.[51] Both IGF-1 and IGFBP3 are provided to wound tissues by plasma and platelets.[52,53] At the wound site, IGF-1 supports the early migration of stromal cells into the fibrin clot and stimulates proliferation of fibroblasts and endothelial cells. Thrombospondin, a component of the fibrin-rich gel, is added to the provisional matrix when the wounds are treated with platelet gels. Thrombospondin, which binds to IGFBP5, increases the action of IGF-1 on cells.[54]

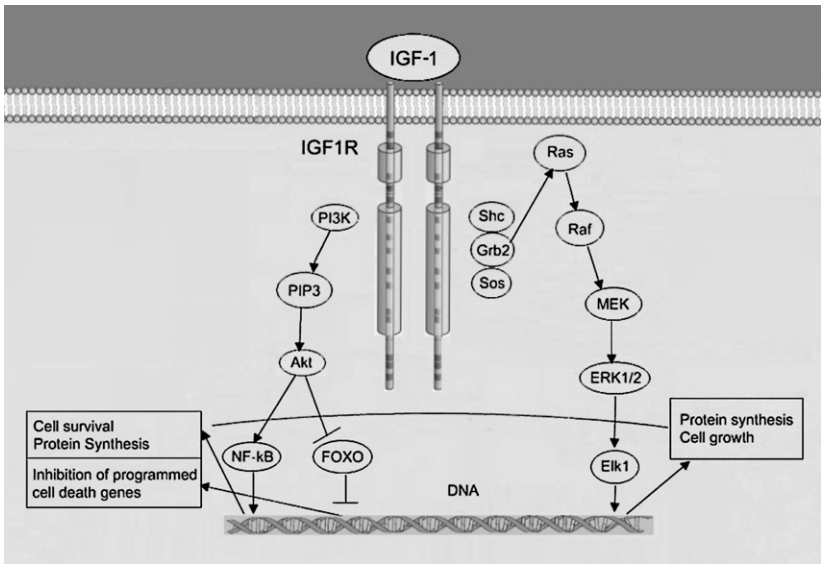

Fig 8. The IGF-1 signaling. Insulin-like growth factor-1 binding to its cell membrane receptor (IGF1R) activates multiple downstream signal transduction cascades recruiting the usual RTK kinases and their intermediates such as Ras, Raf, MEK, ERK, Elk, and the PI3K-Akt pathway. These pathways are involved in cell growth and inhibition of apoptosis. The PI3K-Akt pathway triggers 2 major downstream effector mechanisms at the gene transduction level. One, mediated by activation of the transcription factor NF-κB activation, leads to protein synthesis; the other mediated by inhibition of the transcription factor FOXO, leads to cell survival through inhibition of apoptosis.

Other modulation mechanisms of IGF-1 signaling have been described that belong to the relationships among cells, growth factor network, and context signals. For example, in fibroblasts, all factors able to activate Akt/PKB and, to a lesser extent, ERK1/2, induce the release of IGF-1 that, in turn, may participate in an autocrine loop to sustain the activation of ERK1/2, thus, eliciting DNA synthesis and fibroblast proliferation.[55]

WOUND HEALING

Tissue repair requires synergism and redundancy among the various mechanisms to elicit suitable and stable results. In general, the cellular activities are in response to various signals and each signal can trigger more than one activity. Signal redundancy is the rule in tissue development and repair. Cross talk among the effectors of the multitude of signal transduction cascades have evolved as the homeostatic regulator of signal propagation. Very likely cross talk between the different signal transduction pathways guarantees adaptability that an organism must possess to survive in a constantly changing environment. In this sense, cross talk between the different signaling pathways creates a comprehensive view of the outside world, so that the cell can coordinate these pieces of information and respond in an accurate, efficient, and balanced manner.[56] The coordination of the various signals coming from the PDGFs is facilitated by the fact that most of receptors involved are RTKs (Table 2) making tyrosine kinase activation an integral part of cross talking and of the effective wound healing process.

In normal skin, the basal keratinocytes are attached to the basal lamina through $\alpha 6\beta 4$ integrins connected to cytoskeletal keratin. In wounded skin, keratinocytes must migrate to close the gap. In doing so, the $\alpha 6\beta 4$ integrins are replaced by other integrins, namely the $\alpha 5\beta 1$ vitronectin receptor, and the $\alpha v\beta 6$ fibronectin:tenascin receptors, while relocalizing $\alpha 2\beta 1$ collagen receptors. Cell migration through the fibrin clot requires dissolution of the fibrin barrier by way of plasminogen activators and metalloproteases. Protease up-regulation occurs via ECM-derived signals, via PDGF signals (eg, VEGF, bFGF), and via platelet- and plasma-derived fibronectin.[57] Growth factors belonging to the EGF family (EGF; heparin bound epidermal growth factor, HB-EGF) act as mitogen as well as migration inducer on keratinocytes, fibroblasts, and endothelial cells.

Several factors including TGFβ and PDGF regulate wound repair activity either as mitogen and/or as chemotactic factor for both fibroblasts and inflammatory cells. Similar to keratinocytes, fibroblasts down-regulate their collagen receptors and up-regulate integrins that bind fibronectin and vitronectin to crawl into the provisional fibrin matrix. Transforming growth factor β and mechanical cues induce wound fibroblasts to transform into myofibroblasts. Transforming growth factor β and its downstream effector through early gene induction CTGF, activate fibroblasts, which are induced to produce collagen-rich matrix. Exceeding TGFβ/CTGF activity is implicated in fibrosis and keloid formation. One important difference between fetal and adult healing is the level of TGFβ in the wound site after injury. It is transient and low in the fetus, whereas it is long lasting and at high level in adults.[33] In both fetal and adult injuries, almost identical cytoskeletal machinery is used. In the embryo, repair occurs perfectly, whereas in adult tissue, repair occurs with a variable degree of scarring, possibly owing to inflammation that is virtually absent in fetal life.[8]

Wound healing thus involves complex interactions of several cell types, soluble mediators, ECM molecules, incoming chemoattracted cells, and infiltrating leukocytes. The cellular responses involve direct cell to cell and cell to ECM interactions, as well as indirect cross talk between cell populations via soluble mediators. Inflammatory cells are attracted at the wound site by various soluble factors including interleukin (IL)-1β, tumor necrosis factor-α, interferon-γ; IL-8, PDGF, TGFβ, and many others. In turn, recruited polymorphonuclear cells release several substances including ROS, cationic peptides, and several classes of proteases. Monocyte-macrophages are also attracted by various factors present. Besides their phagocyte and immune functions, macrophages favor the healing outcome through the synthesis of several factors including TGFβ, bFGF, PDGF, and VEGF. Resolution of the inflammatory phase is induced by antiinflammatory cytokines such as IL-10 and TGFβ and by some metalloproteases.[58]

Wound repair in mammals is evolutionarily optimized for speed of healing even under unsanitary conditions. Rapid and redundant inflammatory response allows the wound to heal quickly without infection. In mammals, wound repair and inflam-

mation are very much involved in scarring. Perpetuation of scarring mechanisms may lead to hypertrophic scars and keloids. The quality of scarring (normal or hypertrophic) reflects the variability of inflammation and of the antiinflammatory cytokine profile (including IL-10 and TGFβ) that play a significant role in the modulation of scarring. There are several lines of evidence for how dysregulated inflammation or dysregulation at the interface between inflammation and growth factor network (eg, imbalance of cytokine and metalloprotease network) are associated with 2 undesired conditions: generation of hypertrophic scars and development of chronicity.

Extracellular matrix production by fibroblasts is induced by TGFβ, the intracellular signal being mediated primarily by Smad family of proteins (see Fig 6). In Smad3, deficient mice matrix deposition is retarded; however, wound healing is paradoxically accelerated. In these mice, deficient ECM deposition is reversed by the administration of exogenous TGFβ.[40] Three major points derive from these findings: (1) inflammation is not strictly necessary for reepithelialization and it has probably evolved as a selective advantage against local infections, (2) TGFβ is required to orchestrate fibroblast matrix deposition and repair, and (3) the administration of exogenous growth factors is able to reverse critical conditions hampering normal healing.

Connective tissue growth factor, a downstream mediator of TGFβ released from platelet α-granule during wound healing, is a matricellular protein that can interact with multiple cell surface receptors in a context and cell-specific manner. Connective tissue growth factor controls diverse cell functions such as matrix production, cell proliferation, and cell migration. Connective tissue growth factor can induce fibrosis via fibronectin production through $\alpha v\beta 3$ integrins-mediated signaling pathways that involve Src kinase and subsequent activation of both the PI3K and p42/44 MAPK pathways.[41]

Fibroblasts in chronic ulcers respond poorly to PDGF, bFGF, and EGF, and such responses are not due to down-regulation of growth factor receptors. Inability of wound fibroblasts to pass from G_1 to the S phase of the cell cycle in response to PDGF might be due to (*a*) exhausted proliferation capacity (50 replications per cell) because of repetitive replication induced by mitogenic cytokines under chronic local inflammation, (*b*) loss of local renewal of fibroblast cell population owing to inhibition of chemotactic agents, and (*c*) impairment of intracellular signal transduction of mitogenic factor stimulation.[59] Such inability might also be due to as yet unidentified cell growth-suppressive agents that had been demonstrated in chronic wound fluid. These agents have been shown to down-regulate the level of intracellular Ras, a common downstream effector of several signaling pathways associated with cell survival, growth, and proliferation (see Figs 1, 2, 7, 8).[60]

CONCLUSIONS

Music is a language that communicates spirituality. In Bach's cantatas, the contrapuntal complexity is the structure of delight. Both kind of languages (music and speaking/reading) share common cerebral areas and syntactic-like structures that become activated when music or language are perceived and analyzed.[61] Similar to these languages, linguistic-like syntactic structures underlie the general mechanisms of signal propagation among cells that can download signal transduction within the cell, from membrane to nucleus, and backward. Wound healing is regulated by a well-orchestrated panoply of cytokines, growth factors, and their receptors leading to finely tuned symphony of cell responses (from proliferation to cycle arrest or to apoptosis, from change of cell surface adhesion molecules to migration, and others). Redundancy and iteration of both agonists and modulators are involved in tissue healing, similar to those that exist between instruments and voices. In accepting such a metaphor, we can learn something about grammar and syntaxis of this paradigmatic language. Yet, we perceive the general structure of the language, and we are able to read some phrases. Unfortunately, we are not yet able to correct wrong paragraphs or reverse the biologic consequence of grammatical errors. Nevertheless, learning from the details of languages, we understand that in certain cases of disturbed or disrupted homeostatic equilibrium among healing actors, some healing functions can be rehabilitated via local administration of wild-type platelet-derived factors (in the form of platelet gel or platelet lysate), multiple factor treatment being more effective than single factor administration.[62] Contradicting data are available in the literature with regard to the efficacy of platelet-rich-plasma (PRP) in wound healing.

Recently, it has been reported that the PRP injection in patients with Achilles tendinopathy did not result in greater improvement in pain and activity as compared to placebo injected with saline.[63] These results indicate that the activity of platelet-derived factors present in PRP could depend on various aspects such as the quality of the PRP, quality of wound, type of wound, physiologic conditions of the patients such as age, other ailments that the patients might have, and the intake of prescription drugs by the patients, and others. Therefore, it is premature to conclude that the variable results obtained using PRP are due to its inefficacy. A large number of randomized clinical trials are essential to establish the importance and role of PRP under diverse conditions. In this article, we have highlighted the importance of platelet-derived factors and the complex signaling mechanisms they induce during the course of certain types of normal and chronic wound healing processes. Currently, the understanding of the mode of action of platelet-derived factors and their signaling pathways in tissue repair and regeneration is still at its infancy. Further studies on the precise mechanisms of these factors either alone or in various combinations and concentrations are essential to understand their significance and to use them effectively.

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