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Angiogenesis in Wound Healing

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1. Introduction

Angiogenesis, the formation of new blood vessels from pre-existing, vessels is a crucial process for tumor growth and metastasis (Folkman 1990; Kaafarani, Fernandez-Sauze et al. 2009). The new vessels supply the tumor cells with nutrients and oxygen and ensure efficient drainage of metabolites. Under normal conditions, a tissue or tumor cannot grow beyond 1 to 2 mm in diameter without neovascularization. This distance is defined by limits in the diffusion of oxygen and metabolites, such as glucose and amino acids (Folkman 1971).

In addition to supplying nutrients for tumor growth, angiogenesis is also a gateway for tumor cells and signals to the bloodstream. This direct communication with the bloodstream is essential for the dissemination and metastasis of cancer. After their arrival and deployment in distant organs, metastatic cells again induce angiogenesis in order to support tumor growth (Eichhorn, Kleespies et al. 2007).

As well as this important role of angiogenesis in tumor growth, the whole process of tissue regeneration depends on a new intake of oxygen and metabolites. Growth of new cells for regeneration involves a large energy demand that occurs for the process of cellular mitosis. Therefore, understanding the biochemical mechanisms involved in angiogenesis is necessary for developing interventions in complex tissue regeneration processes.

Since the hypothesis proposed by the surgeon Judah Folkman in the early 70's, which indicated that the inhibition of angiogenesis as a therapeutic target that could halt or even reduce tumor growth (Folkman 1971), intense and successful research on the molecular mechanisms of angiogenesis tumor began. In recent decades, numerous pro- and anti-angiogenic molecules, as well as their ligands and intracellular signaling pathways, have been identified.

2. The wound healing process

The main aim of wound treatment is achieving a rapid closure of the lesion combined with a functional and aesthetically satisfactory scar. To improve current practice, it is essential to gain a better understanding of the biological processes involved in wound healing and tissue regeneration. Many studies have investigated the complex process of wound repair, and the cell behaviors, chemical signals and extracellular matrices that together lead to scarring.

With the disruption of tissue integrity in vertebrates, so begins the repair process, which comprises a sequence of molecular events that either restores or at least secures the damaged tissue. After birth, the body loses its ability to replace damaged tissue without leaving a scar. Only during the fetal stage of life does repair of damage occurs without scar formation, with a true restoration of tissue by a neoformation process (Martin and Leibovich 2005).

Healing has been conveniently divided into three phases, that overlap temporally: the inflammatory, proliferative and remodeling phases (Mendonca and Coutinho-Netto 2009), as shown in figure 1.

2.1 Inflammatory phase (Latent)

After the occurrence of an injury, tissue begins to leak blood that fills the injured area with plasma and cellular elements, mainly platelets. Platelet aggregation and blood clotting generate a plug rich in fibrin; this, in addition to restoring hemostasis and form a barrier against invading microorganisms, organizes a provisional matrix necessary for cell migration. This matrix will also cache growth factors required during the next stages of the healing process (Werner and Grose 2003; Eming, Krieg et al. 2007).

Platelets, essential to the formation of a hemostatic plug, also secrete multiple mediators into the injured area. Platelets are essential in the coagulation cascade, and undergo degranulation induced by thrombin, releasing growth factors, such as platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), epidermal growth factor (EGF), transforming growth factor-α (TGF-α), vascular endothelial growth factor (VEGF) and a adhesive glycoproteins such as fibronectin and thrombospondin, which are important constituents of the provisional extracellular matrix (Streit, Velasco et al. 2000; Nguyen, Hoang et al. 2009; Ribatti 2009). In fact, the coagulation cascade and growth factors released by platelets, together with the activation of the complement cascade and activation of parenchymal cell by injury, produce numerous vasoactive mediators and chemotactic factors, which together assist in the recruitment of inflammatory cells to the wound (Delavary, van der Veer et al. 2011).

In addition to phagocytosing bacteria, cellular debris and foreign bodies, these inflammatory cells produce growth factors that prepare the wound for the proliferative phase, at which time fibroblasts and endothelial cells will continue to be recruited (Singer and Clark 1999; Mendonca and Coutinho-Netto 2009).

Despite the overlap of the healing phases, there is a basic sequence of events: plasma soluble and cellular components exit vessels, followed by platelets, neutrophils and monocytes (Delavary, van der Veer et al. 2011). Subsequently, many neutrophils adhere to the endothelium and migrate to the region of the wound. However, depletion of neutrophils in the blood does not significantly affect the repair process (Simpson and Ross 1972; Werner and Grose 2003; Eming, Werner et al. 2007).

Peripheral blood monocytes, both initially and throughout the course of the healing process, continue to infiltrate the wound in response to chemotactic agents for monocytes, such as PDGF. In the tissue, monocytes are activated and transform into macrophages, which are

probably the main cells involved in control of the repair process (Singer and Clark 1999; Delavary, van der Veer et al. 2011).

Macrophage activation has implications for various aspects of wound healing, as in the phagocytosis of cellular debris, synthesis of extracellular matrix and release of cytokines that stimulate increased vascular permeability, angiogenesis and epithelialization. The release of factors from platelets is the main stimulus for the migration and macrophage activation, while the phagocytosis of cellular components such as fibronectin or collagen also contribute (Henderson, Nair et al. 2011).

The activated macrophage is the main cellular effector in the tissue repair process, degrading and removing damaged tissue components such as collagen, elastin and proteoglycans. As well as removing cellular debris, macrophages secrete chemotactic factors that attract other inflammatory cells to the wound site and produce prostaglandins, which act as potent vasodilators and affect the permeability of microvessels (Singer and Clark 1999; Eming, Werner et al. 2007).

Macrophages produce several growth factors such as PDGF, TGF-β, fibroblast growth factor (FGF) and VEGF, which stand out as the key cytokines necessary to stimulate the formation of granulation tissue. Thus, macrophages mediate the initial phase of the inflammatory response during the wound healing process (Singer and Clark 1999; Barrientos, Stojadinovic et al. 2008).

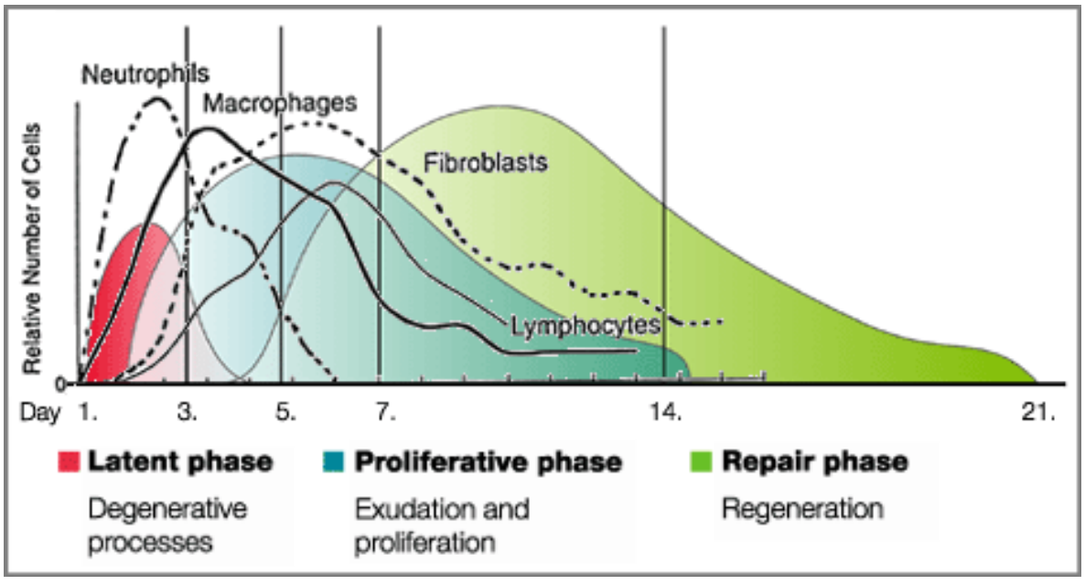


Fig. 1. Migration of immune cell populations correlated with the phases of wound healing

2.2 Proliferative phase

The stage of epithelial proliferation, in the case of the skin, begins with mitogenic and chemotactic stimulation of keratinocytes by EGF and TGF-α. As important as epithelialization, which begins at this stage of the repair process, is the formation of granulation tissue, a name given mainly on account of the characteristic granularity

resulting from the presence of new capillaries. Granulation tissue is essential to repair (Mendonca and Coutinho-Netto 2009).

Before describing angiogenesis, however, it is necessary to note that increased microvascular permeability is the first stage of this process and causes, through the leakage of proteins, cytokines and cellular elements, the formation of a provisional extracellular matrix that is necessary for migration and proliferation of endothelial cells (Dvorak 2002; Dvorak 2010).

2.2.1 Vascular permeability

The production of new blood vessels from pre-existing vessels is accompanied by an increase in vascular permeability (Bates and Harper 2002; Dvorak 2010). In pathological angiogenesis, increased vascular permeability to water and macromolecules is important to the sequence of events that follow injury, being directly responsible for edema. This increased capillary permeability seems to have a minor effect during physiological angiogenesis but it causes considerable damage in pathologies such as diabetic retinopathy (Vaquero, Zurita et al. 2000).

VEGF-A, for example, was discovered in ascites tumor and was originally noticed for its ability to increase the permeability of microvessels and extravasation of macromolecules, including fibrinogen and coagulation proteins, to result in extravascular fibrin deposition which favors both wound healing and tumor development (Dvorak 2010).

The mechanisms of vascular permeability regulation, controlled mainly by growth factors, have not yet been fully elucidated. The function of these growth factors, and the mechanism by which exert their effect, are objects of study of great interest and their metabolic pathways are being elucidated (Dvorak 2005).

2.2.2 Angiogenesis

Angiogenesis is a fundamental step in the healing process by which new blood vessels are formed from preexisting vessels (Folkman and Shing 1992). The new vessels involved in the formation of granulation tissue supply the growing tissue with oxygen and nutrients (Schafer and Werner 2008).

In an adult organism, under normal conditions, angiogenesis occurs only in the reproductive cycle of females (in utero, with the formation of the endometrium and ovaries, with the formation of corpus luteum). Generally, adult vasculature remains quiescent but it has the ability to initiate angiogenesis, especially during healing (Schafer and Werner 2008).

Under physiological conditions, angiogenesis is finely regulated; activated for short periods (days) and then completely inhibited. However, many pathologies are a consequence of lack of regulation, for example, rheumatoid arthritis, where new blood capillaries invade the joint and destroy cartilage. In diabetes, new capillaries present in the retina invade the vitreous humor, bleed, and cause blindness. Tumor growth and metastasis are angiogenesis-dependent diseases, (Folkman 1991). Most tumors remains a constant stimulus to the growth of new capillaries to allow their own growth. The blood vessels also provide a route of communication that allows tumor cells to invade the bloodstream and cause metastases in locations distant from the primary (Folkman and Shing 1992).

The induction of angiogenesis was initially attributed to the acidic or basic FGF. Subsequently, many other molecules have been identified as angiogenic, including VEGF, TGF-β, angiogenin, angiotropin and angiopoetin-1 (Folkman and D'Amore 1996). Low oxygen tension (Detmar, Brown et al. 1997) and high levels of lactate and bioactive amines (Remensnyder and Majno 1968) can also stimulate angiogenesis. Many of these molecules are proteins that induce angiogenesis indirectly by stimulating the production of acidic or basic FGF and VEGF by macrophages and endothelial cells, direct inducers of angiogenesis.

2.2.3 Growth factors

The identification, characterization and purification of VEGF (Vascular Endothelial Growth Factor) in 1989 contributed significantly to understanding the regulation of blood flow and vascular permeability in angiogenesis (Ferrara and Henzel 1989; Glass, Harper et al. 2006). VEGF has three main mechanisms of action: 1) it can increase the vessel permeability to water, small solutes and macromolecules (Adamson, Lenz et al. 2004; Nagy, Benjamin et al. 2008); 2) it can reduce the distance of the tissue cells from to the nearest blood vessel, by stimulating angiogenesis, and 3) it can increase blood flow to tissue by acting as potent vasodilators (Bates and Harper 2002).

VEGF exerts its biological activity predominantly through transmembrane receptors with tyrosine kinase activity present in endothelial cells and participates as a principal mediator of angiogenesis. The VEGF protein family currently includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E and placental growth factor (PlGF) (Werner and Grose 2003). VEGF-A is a homodimer glycoprotein whose subunits are linked by two disulfide bonds, and is synthesized from internal rearrangements ("alternative splicing") of a mRNA, thus there is the production of seven isoforms with 121 to 206 amino acids (Ferrara 2001; Bates and Harper 2002; Ferrara 2004). Among these, the VEGF121, VEGF165, VEGF189 and VEGF206 are the predominant isoforms (Kessler, Fehrmann et al. 2007). These isoforms show similar biological activities, but differ in their binding properties to heparin and extracellular matrix (Roth, Piekarek et al. 2006). The smaller isoforms (121 to 165 amino acids) are secreted in soluble form, while larger ones have transmembrane domains, being initially associated with cells, where they are released and activated by proteolysis. The VEGF121 is an acid protein, while the others have basic isoelectric point.

VEGF is also known as vascular permeability factor (VPF) due to its potent action in increase of vasopermeability, allowing leakage of proteins such as fibrinogen and fibronectin, that are essential for the formation of the provisional extracellular matrix (Nagy, Benjamin et al. 2008), besides increasing the hydraulic conductivity (Bates and Curry 1997) and fenestration (Esser, Wolburg et al. 1998). VEGF also acts as a potent mitogen for endothelial cells of the microvasculature inducing endothelial cell migration and sprouting of new blood vessels through the regulation of several endothelial integrin receptors (Primo, Seano et al. 2010). Furthermore, VEGF also acts as a survival factor for endothelial cells by inducing the expression of Bcl-2, an anti-apoptotic protein (Rao, Zhong et al. 2011).

This family of VEGF exerts its biological functions by differential interactions with three transmembrane receptor tyrosine kinase: VEGF receptor-1 (VEGFR-1) [similar to fms tyrosine kinase (Flt-1)], VEGFR-2 [fetal liver kinase (Flk-1)] and VEGFR-3 (Flt-4). Expression of these receptors is driven primarily by hypoxia. The receptors VEGFR-1 and VEGFR-2 are

restricted to the vascular endothelium, while VEGFR-3, together with its preferred ligand, VEGF-C and VEGF-D seem to be involved in the growth of lymphatic endothelium (Barrientos, Stojadinovic et al. 2008).

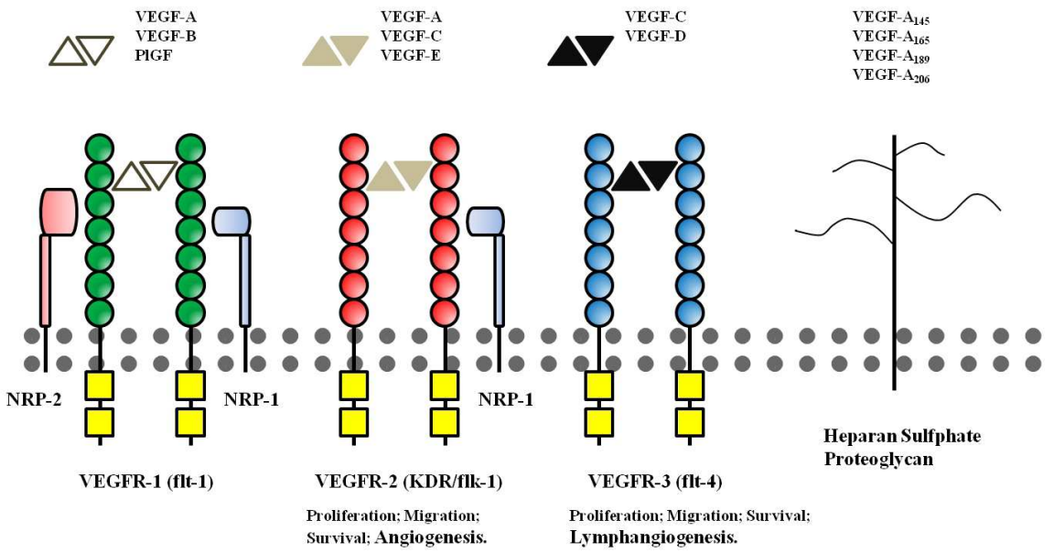


Fig. 2. Vascular endothelial growth factor (VEGF) ligands and receptors. VEGF tyrosine kinase receptors are subfamily of receptor protein tyrosine kinases (RTKs) and possess an extracellular domain containing 7 immunoglobulin-like loops, a single hydrophobic membrane-spanning domain, and a large cytoplasmic domain comprising a single catalytic domain containing all the conserved motifs found in other RTKs. The extracellular domain of VEGFR1 is also independently expressed as a soluble protein (not shown). VEGF-A binds with high affinity to both VEGFR2 (KDR/Flk-1) and VEGFR1 (Flt-1) receptors. Placenta growth factor (PlGF) and VEGF-B exhibit high-affinity binding to VEGFR1 only. VEGF-C and -D are VEGF-related factors that bind to a related receptor, Flt-4 (VEGFR3), and also to VEGFR2. Neuropilin-1 (NRP-1) is a novel non-RTK receptor for VEGF165. Neuropilins and heparan sulfate proteoglycans act as coreceptors that lack enzymatic activity, yet modulate signal output by VEGF receptors.

VEGF is most likely to act through receptors in the endothelium to increase production of nitric oxide (NO) and prostacyclin (PGI2) and augment intracellular endothelial cell survival signaling. NO and PGI2 are predicted to have other biological consequences: decreased platelet aggregation, thrombosis, and, in the case of NO, inhibition of leukocyte adhesion. The combined effect of these biological actions is vascular protection (Zachary 2001).

Many different cell types, fibroblasts, endothelial cells, macrophages and keratinocytes, are able to produce VEGF, and mainly the latter two, are types cells responsible for the production during healing (Barrientos, Stojadinovic et al. 2008). The addition of anti-VEGF inhibits the formation of granulation tissue in the wound (Howdieshell, Callaway et al. 2001) indicating an important function of VEGF in angiogenesis that occurs during the proliferative phase. Low oxygen tension, as occurs during tissue injury, constitutes the greatest inducer of the production of this growth factor (Andrikopoulou, Zhang et al. 2011).

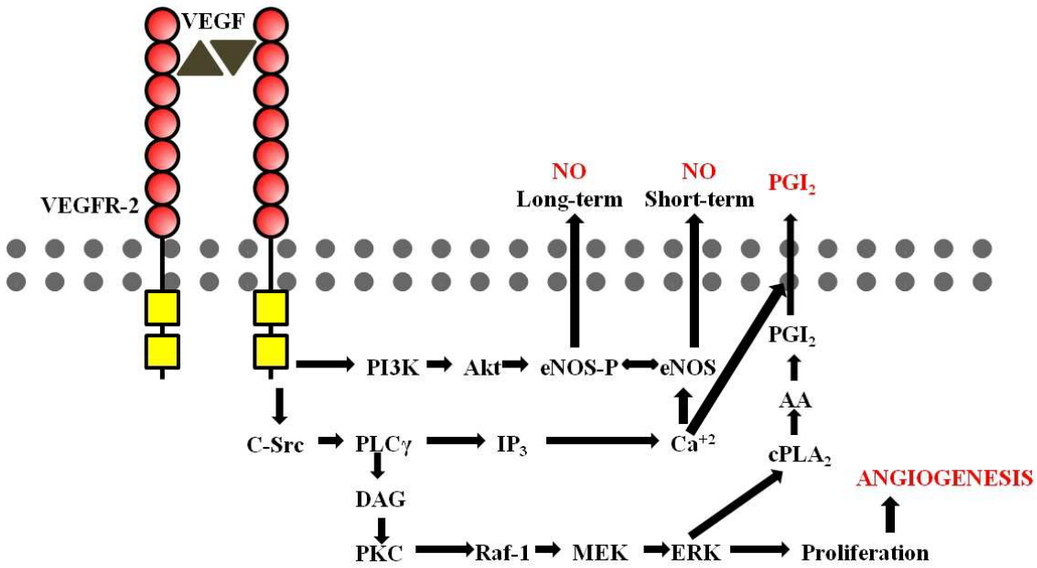


Fig. 3. Mechanisms mediating VEGF-induced NO and PGI2 synthesis. Short-term NO production induced by VEGF is mediated via increased cytosolic Ca^{+2}, resulting from activation of phospholipase C (PLC-gama) and subsequent generation of inositol 1,4,5-trisphosphate (IP_3). c-Src has been implicated in signaling upstream of PLC-γ. Activation of Akt leads to phosphorylation and activation of endothelial NO synthase (eNOS-P), providing a mechanism for sustained Ca^{+2}-independent NO synthesis. PLC-γ -mediated production of diacylglycerol (DAG) leads to activation of PKC, and this pathway plays an important role in mediating VEGF-induced activation of extracellular signal-regulated kinases (ERKs). In turn, ERK activation mediates cytosolic phospholipase A2 ($cPLA_2$)-mediated PGI_2 synthesis. Increased cytosolic Ca^{+2} also stimulates the cellular release of PGI_2 (AA, arachidonic acid).

FGFs are a family of proteins named for their biological activity in promoting the proliferation of fibroblasts in culture. Although the FGF family members follow a numerical designation (Ornitz and Itoh 2001), the designation FGF has only historical value, since FGFs are not only growth factors and their effects are not specifically or universally on fibroblasts. The FGF class of proteins comprises 23 members of homologous structure, all being fairly small polypeptides with a central core containing 140 amino acids. FGF1 (acidic FGF) and FGF2 (basic FGF) are preferentially involved in the process of angiogenesis (Ornitz and Itoh 2001; Barrientos, Stojadinovic et al. 2008). These compounds are polypeptides of about 18 kDa, single chained and non-glycosylated. They transmit their signals through FGF receptor-4 high-affinity, protein family of transmembrane tyrosine kinases (FGFR-1 to FGFR-4), which bind to different FGFs with different affinities. One characteristic of FGF1 and FGF2 is a strong interaction with glycosaminoglycans such as heparan sulfate, present in the extracellular matrix (Folkman, Klagsbrun et al. 1988). This interaction stabilizes FGFs against thermal and proteolytic denaturation, also limit its diffusibility. Thus, the extracellular matrix acts as a reservoir for pro-angiogenic factors. However, neither the use of signal peptide necessary for secretion or release mechanism of these growth factors have been determined to date (Werner and Grose 2003).

Most members of the FGF family act as a broad spectrum mitogen. They stimulate the proliferation of mesenchymal cells of mesodermal origin, as well as ectodermal and endodermal cells. In addition to their mitogenic effects, FGFs regulate the migration and differentiation of their target cells, also showing the cytoprotective function, which increases the survival of cells on adverse conditions (Ornitz and Itoh 2001; Werner and Grose 2003).

The factors FGF1 and FGF2 are synthesized by a variety of cell types involved in angiogenesis and wound healing, including inflammatory cells and dermal fibroblasts. They act on endothelial cells in a paracrine manner, liberated from the extracellular matrix, or in an autocrine way, when released by the endothelial cells themselves, promoting cell proliferation and differentiation. During the formation of granulation tissue, FGF2 promotes cell migration through surface receptors for integrins, which mediate the binding of endothelial cells to extracellular matrix (Barrientos, Stojadinovic et al. 2008).

In addition, many other growth factors and proteins interact during the orchestrated and complex healing process. Proteins such as TGF-beta also act as chemoattractants for neutrophils, macrophages and fibroblasts, stimulate the formation of granulation tissue, demonstrating its importance throughout the healing process. TGF-beta is an important modulator of angiogenesis during wound healing by regulating cell proliferation, migration, capillary tube formation and deposition of extracellular matrix (Brunner and Blakytny 2004; Verrecchia and Mauviel 2007).

2.2.4 Extracellular matrix

For the occurrence of endothelial cell migration and development of new tubular capillaries there is a dependence, not only on cells and cytokines present, but also of the production and organization of extracellular matrix components including fibronectin, collagen, vibronectina, tenascin and laminin, both in the granulation tissue and in the endothelial basement membrane. The extracellular matrix is important for normal growth and maintenance of vessels because, in addition to acting as a scaffold to cell migration, also acts as a reservoir and modulator of the release of growth factors such as FGF2 and TGF-β (Ruoslahti and Yamaguchi 1991; Brunner and Blakytny 2004).

Proliferation of endothelial cells, adjacent to and within the wound, leading to the deposition of the large amounts of fibronectin in the vessel wall (Pankajakshan and Krishnan 2009). Thus, angiogenesis requires the expression of receptors for fibronectin by endothelial cells (Brooks, Clark et al. 1994), organizing fibronectin as a conduit to allow their movement. Expression and activity of proteases are also necessary for angiogenesis, especially during remodeling.

2.3 Remodeling phase (Repair)

At this stage of healing, an attempt is made to recover the normal tissue structure. It is a period marked by maturation of the elements and by changes in the extracellular matrix, resulting in the deposition of collagen and proteoglycans. In a later stage, the fibroblasts of the granulation tissue are transformed into myofibroblasts responsive to contractile agonists that stimulate smooth muscle. As this occurs, a reorganization of the extracellular matrix

takes place, making a final matrix, The balance between the processes that shape this determine the balance between regeneration and scarring (Desmouliere, Chaponnier et al. 2005).

In the process of maturation and remodeling, most vessels, fibroblasts and inflammatory cells disappear from the wound site through migration, apoptosis or other mechanisms of cell death. This leads to the formation of scar with a small number of cells. On the other hand, if the cells persist at the site, the formation of hypertrophic scars or keloids will occur (Mendonca and Coutinho-Netto 2009).

The main cytokines involved in this phase are tumor necrosis factor (TNF-α), interleukin (IL-1), PDGF and TGF-β produced by fibroblasts, and those produced by epithelial cells such as EGF and TGF-β (Karukonda, Flynn et al. 2000).

Re-epithelialization, which covers the wound with new epithelium and involves both migration and proliferation of keratinocytes from the periphery of the lesion, also occurs during the proliferative phase. These events are regulated by three main agents: growth factors, integrins and metalloproteases (Santoro and Gaudino 2005).

During the inflammatory phase, the release of growth factors in the plasma, fibroblasts and macrophages/neutrophils activate keratinocytes located at the margins of the wound. Among the growth factors stand out the PDGF that induces the proliferation of fibroblasts with consequent production of the extracellular matrix during wound contraction and reorganization of the matrix, the keratinocyte growth factor (KGF7) which is considered the main regulator of the proliferation of keratinocytes, and TGF-beta, the principal stimulus for the initial migration of epithelial cells. The activation of integrins by keratinocytes allows cellular interaction with a variety of extracellular matrix proteins in the margin and wound bed. On the other hand, the expression and activation of metalloproteinases promotes the degradation and modification of extracellular matrix proteins in the wound site, facilitating cell migration. The proteolytic activity of these enzymes can release growth factors bound to the extracellular matrix in order to maintain a constant stimulus for proliferation and migration of keratinocytes, accelerating the process of reepithelialization (Santoro and Gaudino 2005).

There are many diseases that interfere with the tissue repair process; they include diabetes, systemic sclerosis, anemia, malnutrition, among others. There are also many conditions that make this process difficult to resolve, preventing or delaying a complete tissue restoration. By obstructing tissue repair, such diseases or conditions potentially contributing to increased morbidity and mortality (Mrué, Coutinho-Netto et al. 2004; Mendonca, Mauricio et al. 2010).

3. Drugs

In recent decades, several studies have been carried out to identify substances capable of promoting the repair process. A search for substances with angiogenic activity has been intense, for their great potential for clinical application.

Among the substances that have direct action in the repair process there are some growth factors that, when applied topically to the wound, demonstrat a good ability to accelerate

tissue repair in animal experiments (Mustoe, Pierce et al. 1991; Pierce, Tarpley et al. 1994). In this group, products based on recombinant human PDGF interfere directly in order to favor the repair process, showing good results in healing of ulcers in diabetic patients (Steed 1998). Other substances containing agents such as enzyme-based ointments DNAse and collagenase act to promote wound debridement (Hebda, Klingbeil et al. 1990) and in this way assist the course of restoration of tissue. The latter are widely used in clinical practice, but have low efficacy in healing chronic wounds. Some angiogenic growth factors and inhibitors are listed on table 1 (Ribatti 2009).

Angiogenic growth factors	**Angiogenesis inhibitors**
Angiogenin	Anastellin
Angiopoietin-1	Angioarrestin
Del-1	Angiostatin
Fibroblast growth factors	Antiangiogenic antithrombin III
Granulocyte colony-stimulating factor	CD59
Hepatocyte growth factor	Chondromodulin
Interleukin-8	Endostatin
Leptin	Heparinases I and III
Midkine	Human chorionic gonadotropin
Placental growth factor	Interferon alfa/beta/gamma
Plasminogen activator inhibitor-1 (low concentrations)	Interferon-inducible protein-10
Platelet-derived endothelial cell growth factor	Interleukin-12
Platelet-derived growth factor	2-methoxyestradiol
Pleiotrophin	Placental ribonuclease inhibitor
Progranulin	Plasminogen activator inhibitor-1 (high concentrations)
Proliferin	Proliferin-related protein
Transforming growth factor-alfa and beta	Retinoids
Tumor necrosis factor-alfa	Tetrahydrocortisol-S
Vascular endothelial growth factor	Thrombospondin-1
	Tissue inhibitors of matrix metalloproteinases
	Troponin
	Vasculostatin and Vasostatin

Table 1. Angiogenic growth factors and inhibitors*

Drug name	Company	Effect on angiogenesis	Indications
Bevacizumab (Avastin)	Genentech	Monoclonal antibody against VEGF-A.	Metastatic coloretal
Ranibizumab (Lucentis)	Genentech	Monoclonal antibody that binds active forms of VEGF-A.	Age-related macular degeneration
Pegaptanib (Macugen)	Pfizer	Selective VEGF inhibitor that binds extracellular VEGF(165)	Age-related macular degeneration
Cetuximab (Erbitux)	Imclone/ Bristol-Myers Squibb	Human-murine monoclonal antibody to EGFR	Metastatic colorectal cancer and squamous cell carcinoma of the head and neck
Panitumumab (Vectibix)	Amgen	Human monoclonal antibody to EGFR	Metastatic colorectal cancer
Trastuzumab (Herceptin)	Genentech	Human monoclonal antibody to HER-2	Adjuvant treatment of HER-2 overexpressing breast cancer and metastasis.
Sunitinib (Sutent)	Pfizer	Inhibitor of multiples RTKs (VEGFRs).	Advanced renal cell and gastrointestinal stromal tumors.
Sorafenib (Nexavar)	Bayer/Onyx	Inhibitor of multiples RTKs (VEGFRs and PDGFR).	Advanced renal cell and inoperable hepatocellular cancers
Erlotinib (Tarceva)	Genentech/OSI	Tyrosine kinase inhibitor of EGFR	Non-small cell lung and pancreatic cancers
Batimastat (British)	Biotech	MMP inhibitor	Vascular stents
Sirolimus/Rapamycin (Rapamune)	Wyeth-Ayerst	mTOR inhibitor, immunosuppressant	Prophylaxis of organ rejection
Temsirolimus (Torisel)	Wyeth	mTOR inhibitor	Advanced renal cell cancer
Everolimus (Xience V)	Abbot Afinitor, Novartis	mTOR inhibitor	Advanced renal cell cancer and vascular stents
Bortezomib (Velcade)	Millenium	Proteassome inhibitor, down regulation VEGF expression	Multiple myeloma and mantle cell lymphoma
Imiquimob (Aldara)	Graceway Pharmaceuticals	Immune modulator, induces production of angiogenic inhibitors	Actinic keratosis, superficial BCC and external genital warts
Thalidomide (Thalomid)	Celgene	Immune modulator, down-regulates expression of bFGF and VEGF	Multiple myeloma anda erythema nodosum leprosum

EGFR –endothelial growth factor receptor; HER-2 – human estrogen receptor 2; RTK – tyrosine kinase receptor; VEGFR – vascular endothelial growth factor receptor; PDGFR – platelet-derived growth factor receptor; MMP – matrix metalloproteinase; mTOR – mammalian targed of rapamycin; BCC – basal cell carcinoma.

Table 2. Antiangiogenics agents approved by FDA (Nguyen, Hoang et al. 2009).

On the other hand, there is much research on substances that could act to inhibit angiogenesis due mainly to their potential for the treatment of cancer. The first angiogenesis inhibitors were reported in the 1980s from the Folkman laboratory (interferon-gamma, administered at low doses). Subsequently, platelet-factor 4, tetrahydrocortisol, and by 1990, a fumagillin analogue were found to have potent antiangiogenic activity.

Angiostatin, an internal fragment of plasminogen, first revealed that an antiangiogenic peptide could be enzymatically released from a parent protein that lacked this inhibitory activity. Endostatin, an internal fragment of collagen XVIII, provided the first evidence that a basement-membrane collagen contained an angiogenesis inhibitory peptide. Thus new drugs with anti-angiogenic activity entered clinical trials. These drugs began to receive U.S. Food and Drug Administration (FDA) approval in the United States by 2003. Bevacizumab was the first angiogenesis inhibitor approved by the FDA (for colon cancer), and the first to demonstrate prolongation of survival in patients with advanced cancer (Folkman 2007). It is an anti-VEGF antibody, and the story of its discovery and manufacture describes a monumental achievement. However, certain non-endothelial cells (haematopoietic-derived cells that colonize tumour stroma and some cancer cells, such as those in pancreatic cancer) can also express receptors for vascular endothelial growth factor (VEGF; also known as VEGFA), raising the possibility that this drug might also have direct anti-tumor effects.

A new target in therapeutic treatment is the hypoxia-inducible factor 1 (HIF-1) an important regulator of cellular response to oxygen deprivation. HIF-1 is a heterodimeric protein that consists of alpha (HIF-1 alpha) and beta (HIF-1 beta) subunits. Under regular oxygen conditions, HIF-1alpha is continuously expressed but is rapidly destroyed by the proteasome pathway. Low oxygen tension results in a decrease in the rate of HIF-1 alpha polyubiquination and proteolysis, and consequent accumulation of the protein. Thus, HIF-1-alpha-HIF-1-beta heterodimers promote angiogenesis, tumor growth, and metastasis regulating the expression of many angiogenic factors. Some studies position mTOR as an upstream activator of HIF-1 function in cancer cells and suggest that antitumor activity of sirolimus (see table 2) is mediated through the inhibition of cellular responses to hypoxic stress (Nguyen, Hoang et al. 2009).

As the treatment range of angiogenesis inhibitors covers not only many types of cancer, but also unrelated diseases such as age-related macular degeneration and possibly others, angiogenesis inhibitors, or drugs that have varying degrees of antiangiogenic activity, might be defined as a class of drugs that specifically target an organizing principle in biomedicine.

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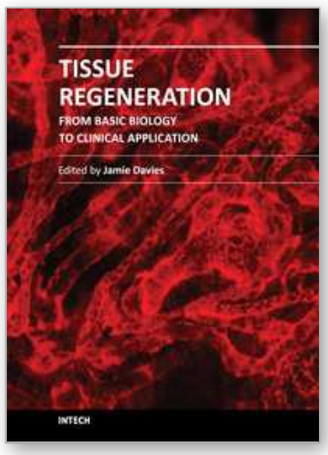
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When most types of human tissue are damaged, they repair themselves by forming a scar - a mechanically strong 'patch' that restores structural integrity to the tissue without restoring physiological function. Much better, for a patient, would be like-for-like replacement of damaged tissue with something functionally equivalent: there is currently an intense international research effort focused on this goal. This timely book addresses key topics in tissue regeneration in a sequence of linked chapters, each written by world experts; understanding normal healing; sources of, and methods of using, stem cells; construction and use of scaffolds; and modelling and assessment of regeneration. The book is intended for an audience consisting of advanced students, and research and medical professionals.

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