Journal of Inorganic Biochemistry 161 (2016) 1–8

Contents lists available at ScienceDirect

Journal of Inorganic Biochemistry

journal homepage: www.elsevier.com/locate/jinorgbio

The neglected role of copper ions in wound healing

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ARTICLE INFO

Article history:
Received 30 October 2015
Received in revised form 19 January 2016
Accepted 10 February 2016
Available online 11 February 2016

Keywords:
Would healing
Copper
Nerve Growth Factor
Metal ion homeostasis
Inflammation
Angiogenesis

ABSTRACT

Wound healing is a complex biological process that aims to repair damaged tissue. Even though many biological and biochemical mechanisms associated with the steps of physiological wound healing are known, there is still significant morbidity and mortality due to dysregulation of physiological mechanisms. It might be useful to revise the activity of old players and their links with new, often neglected, molecular entities. This review revises new findings supporting the hypothesis that copper ions regulate the activity and/or the expression of proteins crucially involved in the wound repair process. A better understanding of these interactions might suggest potential new targets for therapeutic intervention on scars or non-healing wounds.

1. Wound healing

Wound healing is a complex, efficient and highly regulated biological process that aims to repair damaged tissues [1–3]. Skin is a labile tissue with regenerative stem cells in the basal layer of the epidermis [3]. Wound healing begins as soon as tissue injury occurs and it is usually categorized in four sequential, but overlapping, phases: hemostasis, inflammation, proliferation, and remodeling [1,2]. The process requires a well-orchestrated series of responses, resulting in blood clotting, vascular wall repair, protection against infection, cell proliferation and migration, and new blood vessels for the provision of nutrients to those cells [4–6]. These phases utilize a series of perfectly coordinated cellular and molecular events involving numerous biological processes such as proliferation, differentiation, cell migration, and increased biosynthetic activities [3,5,6].

Specific signaling is required to start, modulate and complete the molecular mechanisms occurring during the healing process [3]. Extracellular matrix proteins, growth factors, and cytokines present in the wound bed are fundamental activators of blood coagulation [7–10]. They also contribute to keratinocyte activation, by modifying the function and expression of the adhesion molecules that play a major role in inflammation and subsequent keratinocyte migration [6]. Thus, specific biological markers and biochemical pathways are associated with each step of the physiology of wound healing. However, dysregulation of such mechanisms can occur, resulting in fibrosis and chronic non-healing ulcers, associated with morbidity and mortality due to tissue inflammation and infection [1–4,11,12].

Metal ions are essential catalytic and/or structural elements of many proteins, enzymes, and transcription factors [13–15]. Metal ions are also able to modulate expression level and activity of several proteins through the activation, respectively, of metal-responsive transcription factors and conformational changes [13,15,16]. Data from old and new literature show that the action of many factors involved in the wound healing machinery is modulated by interaction with copper ions [17–31]. Copper has a complex role in various cells, modulating several cytokines and growth factor mechanisms of action, and it is essentially involved in all stages of the wound healing process.

This review will first describe the "classical" pathways activated and involved in the healing process and then will explore the roles of copper ions to interact with and modulate the expression and activity of many proteins and growth factors involved in wound healing machinery.

1.1. Hemostasis

Hemostasis begins from the moment injury occurs, as tissue is disrupted and blood vessels are severed [1,2]. The processes occurring during hemostasis (from Greek αἷμα "blood" and στάσις "stasis")

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http://dx.doi.org/10.1016/j.jinorgbio.2016.02.012
0162-0134/

aim to temporarily seal the damaged tissues and stop associated bleeding, by initiating vasoconstriction, platelet plug formation, and blood coagulation. Vasoconstriction is the first response to the injury. Vasoconstrictive factors such as serotonin and thromboxane A2 act to limit the amount of blood flow through the damaged area [1,32]. Platelets play a major role in hemostasis, undergoing adhesion and aggregation to the damaged endothelium. Platelets form a transient plug to prevent local blood loss [33,34]. Then, platelets themselves provide a surface for coagulation factors such as fibrinogen that, in turn, is converted to fibrin (via the extrinsic and intrinsic coagulation cascade) forming a sturdy blood clot [33,35,36].

The blood clot acts as scaffolding, allowing a temporary extracellular matrix (ECM) to form around the clot in the wound area [8]. The extracellular matrix is rich in fibrous proteins such as collagen, fibronectin and fibrin, as well as liquid proteoglycans like hyaluronic acid [8]. It is important to note that the ECM contributes to wound repair not only structurally but also by signaling, attracting different cells to itself [37]. A major player of the ECM involved in wound healing is fibronectin [38,39]. Fibronectin is a large ECM adhesion glycoprotein that interacts with integrin receptors to stimulate migration and adhesion of fibroblasts, keratinocytes and endothelial cells as well as serving to promote angiogenesis (the physiological process through which new blood vessels are generated from pre-existing vessels) [39]. The temporary matrix envelops platelets as part of the matrix itself. Platelets in the matrix attract and activate numerous cells such as endothelial cells, fibroblasts, neutrophils and macrophages [35,36,39]. Thus, besides acting as a "static" plug, the platelets also importantly pave the way of the next steps of wound healing, by releasing several pro-inflammatory factors (including prostaglandins, prostacyclins, thromboxane, and histamine), and several cytokines and growth factors, including platelet-derived growth factor (PDGF), transforming growth factor beta (TGF-β) and vascular endothelial growth factor (VEGF) [33,35,36].

1.2. Inflammation

Histamine, locally released, is essentially responsible for capillary vasodilatation, which: i) allows increased blood flow, ii) causes blood vessels to become porous and thus iii) increases vascular permeability [1,40,41]. All together, histamine release results in the arrival of inflammatory cells into the wound site, thereby setting the stage for the inflammatory phase [1,40,41].

Neutrophils and macrophages are the main cells involved in the inflammatory reaction in injured tissues [3,42,43]. Their chemotactic attraction to the site of injury is guaranteed by the release of PDGF and TGF-β from activated platelets during the hemostasis phase [33,35,36]. Neutrophils phagocytize debris and kill bacteria by releasing free radicals in what is called a "respiratory burst". This involves the induction of inducible nitric oxide synthase (iNOS) to produce large amounts of nitric oxide (NO) that react with the rapidly released superoxide anion radical to form the highly diffusible peroxynitrite and hydrogen peroxide radicals [44].

Macrophages enter the wound site to remove remaining debris, bacteria, damaged tissue, and apoptotic neutrophils, thus paving the way for the resolution of inflammation [3,42,43]. Macrophages release many factors, including plasminogen activator (to produce plasmin and remove the fibrin clot), cytokines to recruit keratinocytes and stimulate angiogenesis, thus further facilitating the next phase in healing [3,37,42,43]. Pathologies with low levels or activity of monocytes or macrophages lead to poor wound debridement, delayed arrival of fibroblasts, and inadequate proliferation phase and angiogenesis [4,11,32].

1.3. Proliferation

The proliferative phase takes place as soon as the inflammatory phase is over and all debris are cleared. This phase is characterized by granulation tissue, revascularization, and re-epithelialization of the wound [1,2]. The fibroblasts are recruited to the wound site mainly by PDGF and TGF-β secreted by the platelets and macrophages [4,42]. The fibroblasts synthesize the granulation tissue, which contains collagen (types I and III), elastin, proteoglycans, glycosaminoglycans, fibronectin and non-collagenous proteins [8]. Fibronectin is particularly important, as it is organized into fibrillar structures within the stroma of the granulation tissue [38]. It forms a dense network around the fibroblasts that is vital i) for establishing and maintaining tissue architecture, and ii) for regulating cellular processes such as adhesion, spreading, proliferation, migration and apoptosis [37–39].

Angiogenesis also occurs within the proliferative process [45]. Endothelial cells were recruited during hemostasis and the inflammatory phase via multiple chemokines, specifically TGF-α and VEGF, as well as local secretion of the ECM [8,37,45]. The ECM regulates angiogenesis by providing structural support for new vessels, spreading out signals and growth factors for angiogenesis, and having major receptors to mediate interactions needed for angiogenesis [8,45]. Endothelial cells proliferate and begin to differentiate, forming a network of branching vessels in the newly formed granulation tissue formation. This step provides nutrition and oxygen to growing tissues and replaces the vessels that were destroyed in the wound [45]. Clinically, at this stage granulation tissue is said to have a "beefy red" appearance, which is a consequence of endothelial cell division to create capillary networks.

1.4. Remodeling

During the final phase of healing, morphological changes occurr on the wounded skin. The capillaries aggregate to form larger blood vessels resulting in less blood flow, and thus reduced nutrients availability [1,4]. This reflects the decrease in cell density and metabolic activity of the tissue, largely due to apoptosis as a collateral effect of inflammation. In the extracellular matrix an increase of the tensile strength occurs upon increased proportion of collagen type I versus collagen type III that rearrange in a tight organized fashion, with a higher number of covalent cross-links [8,9,45]. Although the exact mechanism is not known, fibroblasts are hypothesized to differentiate into myofibroblasts and produce a high contractile force using alpha-smooth muscle actin [46]. Expression of alpha-smooth muscle is influenced by TGF-β and ECM-specialized proteins like fibronectin [46].

The final result of tissue repair is a scar, which is less elastic than normal skin, does not contain skin appendages such as hair follicles or sweat glands, and has a weaker tensile strength that only reaches about 80% that of unwounded skin [1,2].

2. Nerve Growth Factor and wound healing

Nerve Growth Factor (NGF) is a protein belonging to the neurotrophin family that is involved in cell growth, maintenance, differentiation, survival, and synaptic plasticity of the central and peripheral nervous systems [47,48]. Over time, however, it has been shown that the role and action of neurotrophins is not restricted to the "classical" view of the nervous system. Namely, NGF has been revealed as a molecule with angiogenic properties, as well as having a role in inflammatory and immune responses in non-neuronal tissues, specifically with its function in skin and tissue repair [49,50].

NGF is a secreted protein that triggers its biological action through interaction with two distinct classes of cell-surface receptors: TrkA (tropomyosin receptor kinase A) and 75 kDa neurotrophin receptor (p75). Trk signaling occurs through different pathways, including the extracellular signal-regulated kinases (ERK), phosphatidylinositide 3-kinases/protein kinase B (PI3K/Akt), phosphoinositide phospholipase C gamma (PLCγ) pathway. These pathways affect neurotrophin signaling in terms of cell survival and cell differentiation, while NGF binding to p75 can trigger neuron apoptosis [51].

NGF and NGF receptors are highly expressed in the skin, where NGF is produced by epidermal cells such as fibroblasts, keratinocytes, and