Chapter 3

Biomarkers of Wound Healing

Christian Agyare, Newman Osafo and
Yaw Duah Boakye

Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.80222

Abstract

The prevalence of conditions that eventually result in poor wound healing abounds as humans advance in age. With the increased possibility of wounds not healing comes a leap in morbidity and mortality with its accompanying socioeconomic impact. It is therefore relevant to understand what accounts for aberrant wound healing and more importantly the molecular markers involved in this pathological state. There are known events associated with the wound healing process, spanning from cellular involvement to the role of specific proteins such as cytokines and growth factors that are significant biomarkers in the wound healing process. This chapter discusses biomarkers relevant to the wound healing process, and these biomarkers go a long way to help identify and stratify nonhealing patients for whom biomarker-guided approaches may be of importance clinically in their management.

Keywords: wound, biomarkers, cytokines, growth factors, proteases

1. Introduction

The concept of biomarkers has existed from the time of the inception of ayurvedic medicine, just around the seventh century when the sweetness of urine was linked to diabetes even though the terminology had not been developed then [1]. The perspective of what constitutes the definition of a biomarker is somewhat diverse. Biomarkers (biological markers) are generally biomolecules whose qualitative and quantitative presence provides an indication of the state of a biological system. A more exhaustive definition as provided by the World Health Organization (WHO) led joint venture on chemical safety that describes a biomarker as any substance, structure, or process that can be measured in the body or its products that can

influence or predict the incidence of outcome or disease [2]. The application of biomarkers has attained a vital and grounded position in clinical research, usually as predictors of the clinical outcomes for a varied number of disease conditions and their management [3].

Extensive scientific investigation into the mechanism of wound healing has revealed that the traditional guides in the determination of the wound healing potential, i.e., erythrocyte sedimentation rate (ESR) and C-reactive protein, do not yield enough positive and negative predictive values [4]. In lieu of the scientific evidence available, the focus has shifted to cytokines, chemokines, and proteases which hold the greatest potential as biomarkers [4].

2. Cytokines

Cytokines are proteins of relatively low molecular weight that are secreted to influence or modulate the behavior of immune cells and also other cells [5]. Crucial among them include interleukins, lymphokines, and other signaling molecules such as interferons and tissue necrosis factor (TNF-α). It has been long considered and corroborated by scientific evidence that pro-inflammatory cytokines such as interleukins 1α (IL-1α), 1β (IL-1β), and 6 (IL-6) and TNF-α play essential roles in wound healing process such as the stimulation of keratinocyte and fibroblast proliferation, modulation of immune response, synthesis and breakdown of extracellular matrix proteins, and the chemotaxis of fibroblast to the wound site [6].

Grellner et al. [7, 8] in their work to quantitatively analyze pro-inflammatory cytokines in human skin wounds realized an upregulation of the expression of IL-1α, IL-1β, IL-6, and TNF-α in the inflammatory phase of the wound healing process. The levels of these pro-inflammatory cytokines (TNF-α, IL-1, and IL-6) were higher in nonhealing wounds than healing wounds owing to the fact that nonhealing wounds stay in the inflammatory phase of wound healing process [4]. Bilder et al. [9] also report an increase in the levels of IL-8 in chronic nonhealing wounds as opposed to those with a healing potential. Ligi et al. [10] upon the assessment of several studies which evaluated the level expression of cytokines and chemokines in the microenvironment of a chronic ulcer alluded to a heightened pro-inflammatory condition in a nonhealing wound, thus corroborating other studies. It was however noted that the level of cytokines detectable does not necessarily correlate to its bioactivity due to anti-inflammatory cytokines whose presence counteracts the activity of these pro-inflammatory cytokines [10]. There are also specific cytokine inhibitors and proteolytic enzymes that also act on these cytokines to mask their bioavailability [10]. Patel et al. [4] also report the inconsistency in wound and serum levels of cytokines which poses a challenge in its use as reliable biomarkers of nonhealing wounds.

2.1. Interleukin 1 (IL-1)

The IL-1 family of cytokines is made up of two pro-inflammatory cytokines, namely, IL-α and IL-β. Interleukin 1 is primarily sourced from macrophages in the event of injury, infection, and antigenic challenge although the epidermal, epithelial, lymphoid, and vascular

tissues also serve as reservoirs for the polypeptide [11]. The actions of IL-1 span from systemic changes in the neurological, hematologic, endocrinologic, and metabolic systems to some local effects that are particularly relevant in wound healing [12]. By influencing both destructive and repair processes, it contributes the mesenchymal tissue remodeling, and it does so by influencing quite a number of cells. First of all, it stimulates capillary endothelial cells to produce chemokines such as MCP-1 and also cause an upregulation of the synthesis of vascular adhesion molecules such as ICAM-1, VCAM-1, and E-selectin [13, 14]. The combined effect of these two actions is to cause the infiltration of the injury site with mononuclear cells, thus setting the stage for inflammatory response. The expression of matrix metalloproteases (MMPs) from resident fibroblasts is also under the control of IL-1. The call of MMPs to play results in the degradation of the extracellular matrix to allow for enhanced monocyte migration. It also leads to a down-modulation of the inflammatory response as MMPs degrade IL-1. Inhibiting the IL-1 pathway through the use of recombinant antibodies and macrophages from IL-1 receptor knockout mice appeared to turn the tables around as far as the wound microenvironment is concerned by inducing a switch from pro-inflammatory to healing-associated macrophage phenotypes and growth factors [14]. Therefore, there is a negative implication for wound healing in the absence of high expression of IL-1.

2.2. Interleukin 6 (IL-6)

Interleukin 6 is described as the chief contributor to the stimulation of a majority of the acute-phase proteins during inflammation. IL-6-deficient transgenic mice (IL-6 KO) therefore showed a substantial delayed cutaneous wound healing relative to the wild-type control animals by about threefold, the time required for healing [15].

Based on similar animal model studies on IL-6 knockout mice and the administration of recombinant murine IL-6 protein, IL-6 was found to be essential in stimulating the mitogenic activity of keratinocytes, an action that has been linked to scar formation as well as exerting a chemo-attractive action on neutrophils [6]. These effects seek to kick-start the wound healing process. However, a study conducted to determine the indicators of inflammation in the pathogenesis of diabetic foot ulcers identified a positive correlation between high serum IL-6 levels in diabetic patients with foot ulcers and low serum IL-6 levels in those without foot ulcers. This implicates its effect on poor wound healing [16].

This is not surprising as IL-6 has a reputation for dictating the transition from acute to chronic inflammation systemically by its stimulatory effects on T and B cells.

2.3. Tumor necrosis factor-α (TNF-α)

Tumor necrosis factor alpha (TNF-α) is a key pro-inflammatory cytokine involved in the early phase of most inflammatory events in the body. Employing mouse models, the expression of TNF-α at detectable levels was discovered to happen just after wound creation and sees an increase in the first several hours until it reaches a peak within 24 hours after which it returns to the basal level [17]. Vascular endothelial cells, keratinocytes, and fibroblasts are the major sources of TNF-α which cause an initiation of the inflammatory phase of the wound

healing by promoting the recruitment of inflammatory leukocytes. TNF-α is also involved in the regulation of the activity of fibroblasts, keratinocytes, and vascular endothelial cells as well as in modulating synthesis of extracellular matrix proteins and matrix metalloproteinase [17, 18]. Based on diabetic models, an increase in TNF-α level coupled with decrease in IL-10 that has anti-inflammatory properties results in sustained expression of chemokines CXCL2 and CCL2 and leads to continuous infiltration of leucocytes to the injury site. This ultimately prolongs the inflammation and reduces the wound healing potential [19].

2.4. Transforming growth factor (TGF)

Transforming growth factor describes the superfamily for pluripotent cytokines which have very important functions to perform during disease, homeostasis, development, and repair. These sets of proteins are structurally related, but functionally distinguishable and relevant among them for wound healing are the isoforms TGF-β 1–3 [20]. The roles of these isoforms in the wound healing process can be both distinct and overlapping. However, the overall nature of their contribution to the wound healing has generated some controversy and thus is among the most studied molecules involved in the process [6]. Transforming growth factor β1 (TNF-β1) however has the widest spectrum of actions, affecting all manners of cell types that are involved in all stages of wound healing. These effects have been reported to be both positive and negative [21]. Historically, the synthesis of TNF-β1 from keratinocytes, platelets, and macrophages is upregulated right after injury, and this is crucial for initiating inflammation and granulation tissue formation. In addition, TNF-β1 contributes to the chemotactic migration of cells during wound repair. Some proteases such as MMP-1, MMP-2, MMP-3, and MMP-9 are also under the control of TNF-β1 [6, 22]. Based on human studies, TNF-β1 was found to stimulate the production of extracellular matrix molecules, including collagens and fibronectin, which strengthen the repaired wound. In spite of this knowledge, available evidence goes to raise questions about the true effects of TNF-β1 levels on wound healing [23]. Wound healing in Smad knockout mice, which have the signaling pathway of TNF-β1 blocked, was rather accelerated to the surprise of the investigators. In similar fashion, TNF-β1 knockout mice showed demonstrated reepithelialization during incisional wound repair, in comparison with wild-type mice. The consensus in the face of current evidence is that the selective inhibition of TNF-β1 in some cells may prove beneficial [24].

3. Growth factors

The growth factors are essentially responsible for the initiation of the proliferation stage of the wound healing process. The platelet-derived growth factor (PDGF), transforming growth factors (TGF-α, TGF-β), insulin growth factor (IGF-1), fibroblast growth factor (FGF), and granulocyte-macrophage colony-stimulating factors (GM-CSF) are examples of growth factors whose roles in wound healing as well as their possible use as biomarkers have been studied extensively based on their expressed levels [25]. In spite of the fact that insight about ideal levels and the spatiotemporal distribution of growth factors is far from complete, available data

points to no local growth factor deficiency in chronic leg ulcers with the possible exception of TGF [6]. Trengove et al. [26] after studying wound fluids from both healing and nonhealing wounds arrive at similar conclusion that poor wound healing may be due to inflammatory mediators rather than a deficiency of growth factors.

3.1. Platelet-derived growth factors (PDGF)

Platelet-derived growth factors (PDGFs) are made up of a family of homodimeric or heterodimeric growth factors, including PDGF-AA, PDGF-AB, PDGF-BB, PDGF-CC, and PDGF-DD [27]. PDGF has been established to have chemotactic role for cells that migrate to the healing wound site such as fibroblasts, neutrophils, and monocytes. It was actually the very first growth factor shown to have this function [28]. It additionally stimulates the proliferation of fibroblast and the deposition of extracellular matrix. In vitro studies have also revealed that it stimulates insulin growth factor (IGF) release in fibroblasts which is vital to the initiation of the repair process [28]. Lastly, it stimulates fibroblasts to contract collagen matrices and induces the myofibroblast phenotype in the implicated cells. It has thus been established to be a major player in the wound healing and has formed the basis for studies into its clinical application in the treatment of wound healing disorders.

Owing to the close proximity of the expression sites of the PDGF, which is predominantly in the epidermis, and its receptors which are also in the dermis and granulating tissue, a paracrine mechanism has been suggested for its action [6, 29]. However, unlike other growth factors like fibroblast growth factor (FGF) and vascular epithelial growth factor (VEGF) that see an overexpression in the microenvironment or at the site of a healing wound or one in a granulation phase, the increase in the expression of PDGF-BB is without this spatial limitation as its levels in plasma also increases. It does make it potentially useful as the biomarker in wound healing [10].

4. Proteases

The action of proteases and their inhibitors goes a long way to influence the equilibrium between extracellular matrix (ECM) degradation and deposition which is responsible for the coordinated and timely healing of wounds [30]. There is an overwhelming wealth of evidence to suggest that nonhealing wounds are characterized by an increase in the levels of proteases and an imbalance in the protease/protease inhibitor levels [30, 31]. This manifests as a persistence of proteolysis and degradation of the extracellular matrix causing wound healing to delay. Significant among these proteases are the matrix metalloproteases (MMPs) [32]. MMPs are part of a family of zinc endopeptidase which essentially help in the degradation of provisional extracellular matrix, facilitate the migration of inflammatory cells to the wound site, remodel the granulation tissue, and modulate angiogenesis [28]. MMP activity as measured using Azocoll assay was found to be significantly elevated in chronic wounds as compared to acute wounds, thus implicating it poor wound healing [26].

Proteases as biomarkers for wound healing hold the key to transform clinical approach to the management of wounds. For example, the appropriateness of using protease-modulating dressing and tissue-engineered products, scaffolds, and skin grafts for the treatment can be made by the determination of the levels of proteases [33].

5. Matrix metalloproteinase

Matrix metalloproteinases (MMPs) are a group of endopeptidase that are zinc and calcium dependent and are usually divided into six groups depending on the substrate they act on. These MMPs consist of collagenases (MMP-1, MMP-3, MMP-8); gelatinases (MMP-2, MMP-9); stromelysins (MMP-3, MMP-10); matrilysins (MMP-7, MMP-26); membrane-type MMPs (MT-MMP) like MMP-14, MMP-15, MMP-16, and MMP-24; and other MMPs (MMP-11, MMP-12, MMP-19, MMP-20, MMP-22, MMP-23, MMP-28) [34].

Various MMPs are relevant to the wound healing process at varied points, and the tight control of their proteolytic activity is also essential to conduct the different events of wound healing [36]. MMPs are however generally involved in the inflammatory, proliferative, and remodeling phases of the wound healing process by modulating cytokine/chemokine activity by activating them enzymatically or influencing their availability by cleaving them from cell surface. Additionally, the actions of MMPs involve the breakdown of proteins part of the cell-cell and cell-extracellular matrix interaction [35, 36].

In terms of the predictive roles of MMPs' level for the wound healing process, some studies have focused on the MMP-1 to tissue inhibitor of metalloproteinase (TIMP-1) ratio. In one study, for instance, a significant correlation was found between a high ratio of MMP-1/TIMP-1 and good healing ($r = 0.65$, $p = 0.008$) with receiver operator curve (ROC) analysis showing an MMP-1/TIMP-1 ratio of 0.39 being the best predictive value for wound healing. High levels of MMP-8 and MMP-9 also appear to have negative predictive value for the process of wound healing [32].

6. Conclusion

With the growing research into the therapeutic benefits of biomarkers comes the challenge of identifying biomarkers that satisfy the required characteristics for use clinically. It is prudent to validate new biomarkers affecting the wound healing process by employing innovative, simple, and cost-effective molecular approaches to determine the type, level, and activity of all potential biomarkers. With the advent of trendsetting technical knowhow in defining diseases and other biological processes, it has become increasingly possible to identify and characterize novel biomarkers of the wound healing process. Continuing the research into identification of new biomarkers affecting the wound healing process is imperative since it will eventually have weighty health benefits on patients and offer a relevant guide to wound management. This will significantly lower the risks of microbial colonization and invasion of wounds and loss of structural function as a result of chronic wounds.

Author details

Christian Agyare[1]*, Newman Osafo[2] and Yaw Duah Boakye[1]

*Address all correspondence to: cagyare.pharm@knust.edu.gh

1 Faculty of Pharmacy and Pharmaceutical Sciences, Department of Pharmaceutics, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

2 Faculty of Pharmacy and Pharmaceutical Sciences, Department of Pharmacology, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

References

[1] Karley D, Gupta D, Tiwari A. Biomarkers: The future of medical science to detect cancer. Journal of Molecular Biomarkers Diagnosis. 2011;**2**:118

[2] WHO International Programme on Chemical Safety Biomarkers in Risk Assessment: Validity and Validation. 2001. Retrieved from: http://www.inchem.org/documents/ehc/ehc/ehc222.htm

[3] WHO International Programme on Chemical Safety Biomarkers and Risk Assessment: Concepts and Principles. 1993. Retrieved from: http://www.inchem.org/documents/ehc/ehc/ehc155.htm

[4] Patel S, Maheshwari A, Chandra A. Biomarkers for wound healing and their evaluation. Journal of Wound Care. 2016;**25**(1):46-55

[5] Cytokine. In: Stedman's Medical Dictionary. 28th ed. Wolters Kluwer Health, Lippincott: USA: Williams & Wilkins, Philadelphia; 2006

[6] Werner S, Grose R. Regulation of wound healing by growth factors and cytokines. Physiological Reviews. 2003;**83**(3):835-870

[7] Grellner W, Georg T, Wilske J. Quantitative analysis of proinflammatory cytokines (IL-1beta, IL-6, TNF-alpha) in human skin wounds. Forensic Science International. 2000;**113**:251-264

[8] Grellner W, Vieler S, Madea B. Transforming growth factors (TGF-alpha and TGF-beta1) in the determination of vitality and wound age: Immunohistochemical study on human skin wounds. Forensic Science International. 2005;**153**:174-180

[9] Bilder D, Schober M, Perrimon N. Integrated activity of PDZ protein complexes regulates epithelial polarity. Nature Cell Biology. 2003;**5**:53-58

[10] Ligi D, Mosti G, Croce L, Raffetto JD, Mannello F. Chronic venous disease—Part I: Inflammatory biomarkers in wound healing. Biochimica et Biophysica Acta. 2016;**1862**(10): 1964-1974

[11] Chamberlain SC, Leiferman ME, Frisch EK, et al. Interleukin expression after injury and the effects of interleukin-1 receptor antagonist. PLoS One. 2013;**8**(8):e71631

[12] Doersch KM, DelloStritto DJ, Newell-Rogers MK. The contribution of interleukin-2 to effective wound healing. Experimental Biology and Medicine. 2017;**242**(4):384-396

[13] Cook-Mills JM, Marchese ME, Abdala-Valencia H. Vascular cell adhesion molecule-1 expression and signaling during disease: Regulation by reactive oxygen species and antioxidants. Antioxidants and Redox Signaling. 2011;**15**(6):1607-1638

[14] Lobmann R, Zemlin C, Motzkau M, Reschke K, Lehnert H. Expression of matrix metalloproteinases and growth factors in diabetic foot wounds treated with a protease absorbent dressing. Journal of Diabetes and its Complications. 2006;**20**(5):329-335

[15] Gallucci RM, Simeonova PP, Matheson JM, Kommineni C, Guriel JL, Sugawara T, Luster MI. Impaired cutaneous wound healing in interleukin-6–deficient and immunosuppressed mice. FASEB Journal. 2000;**14**(15):2525-2531

[16] Sallam AWA, El-Sharawy MHA. Role of interleukin-6 (IL-6) and indicators of inflammation in the pathogenesis of diabetic foot ulcers. Australian Journal of Basic and Applied Sciences. 2012;**6**(6):430-435

[17] Ritsu M, Kawakami K, Kanno E, Tanno H, Ishii K. Critical role of tumor necrosis factor- a in the early process of wound healing in skin. Journal of Dermatology and Dermatologic Surgery. 2017;**21**(1):14-19

[18] Mast BA, Schultz GS. Interactions of cytokines, growth factors, and proteases in acute and chronic wounds. Wound Repair and Regeneration. 1996;**4**:411-420

[19] Sen CK. Wound healing essentials: Let there be oxygen. Wound Repair and Regeneration. 2009;**17**:1-18

[20] Huang T, Schor LS, Hinck PA. Biological activity differences between TGF-β1 and TGF-β3 correlate with differences in the rigidity and arrangement of their component monomers. Biochemistry. 2014;**53**(36):5737-5749

[21] Wang XJ, Han G, Owens P, Siddiqui Y, Li AG. Role of TGF beta-mediated inflammation in cutaneous wound healing. The Journal of Investigative Dermatology. Symposium Proceedings. 2006;**11**(1):112-117

[22] Manicone AM, McGuire JK. Matrix metalloproteinases as modulators of inflammation. Seminars in Cell and Developmental Biology. 2008;**19**(1):34-41

[23] Goncalves MBB, Rabeh SAN, Tercariol CAS. The contribution of distance learning to the knowledge of nursing lecturers regarding assessment of chronic wounds. Revista Latino-Americana de Enfermagem. 2015;**23**(1):122-129

[24] Tejiram S, Kavalukas SL, Shupp JW, Barbul A. Wound healing. Wound Healing Biomaterials. 2016:3-39

[25] Olczyk P, Mencner Ł, Komosinska-Vassev K. The role of the extracellular matrix components in cutaneous wound healing. BioMed Research International. 2014;**2014**:747584, 8 pages

[26] Trengove NJ, Stacey MC, Macauley S, et al. Analysis of the acute and chronic wound environments: The role of proteases and their inhibitors. Wound Repair and Regeneration. 1999;**7**(6):442-452

[27] Rönnstrand L. Signaling by the platelet-derived growth facto receptor family. In: Bradshaw RA, Dennis EA, editors. Handbook of Cell Signaling. Cambridge, Massachusetts, USA: Academic Press. p. 427

[28] Diagn JMB, Gupta A. Wound healing: Assessment by various markers. Journal of Molecular Biomarkers. 2016;**8**(2):8-10

[29] Pastar I, Stojadinovic O, Yin NC, et al. Epithelialization in wound healing: A comprehensive review. Advances in Wound Care. 2014;**3**(7):445-464

[30] McCarty SM, Percival SL. Proteases and delayed wound healing. Advances in Wound Care. 2013;**2**(8):438-447

[31] Demidova-Rice TN, Hamblin MR, Herman IM. Acute and impaired wound healing: Pathophysiology and current methods for drug delivery, part 1: Normal and chronic wounds: Biology, causes, and approaches to care. Advances in Skin & Wound Care. 2012;**25**(7):304-314

[32] Iuonut AM, Dindelegan GC, Ciuce C. Proteases as biomarkers in wound healing. Temporomandibular Journal. 2011;**61**(1-2):65-73

[33] International Consensus. The Role of Proteases in Wound Healing Diagnostics. An Expert Working Group Review. London: Wounds International; 2011. p. 10

[34] Ligi D, Mosti G, Croce L, Raffetto JD, Mannello F. Chronic venous disease—Part II: Proteolytic biomarkers in wound healing. Biochimica et Biophysica Acta. 2016;**1862**(10): 1900-1908

[35] Caley MP, Martins VLC, O'Toole EA. Metalloproteinases and wound healing. Advances in Wound Care. 2015;**4**(4):225-234

[36] Jablonska-Trypuc A, Matejczyk M, Rosochacki S. Matrix metalloproteinases (MMPs), the main extracellular matrix (ECM) enzymes in collagen degradation, as a target for anticancer drugs. Journal of Enzyme Inhibition and Medicinal Chemistry. 2016;**31**(1):177-183

IntechOpen