BBA - Molecular Cell Research 1864 (2017) 2220–2227

Contents lists available at ScienceDirect

BBA - Molecular Cell Research

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

Matrix metalloproteinases: The sculptors of chronic cutaneous wounds☆

Venkat Raghavan Krishnaswamy[1], Dvir Mintz[1], Irit Sagi*

Department of Biological Regulation, Weizmann Institute of Science, Rehovot, 7610001, Israel

ARTICLE INFO

Keywords:
Chronic wound
Extracellular matrix
Infection
Matrix metalloproteinase
Wound healing

ABSTRACT

Cutaneous wound healing is a complex mechanism with multiple processes orchestrating harmoniously for structural and functional restoration of the damaged tissue. Chronic non-healing wounds plagued with infection create a major healthcare burden and is one of the most frustrating clinical problems. Chronic wounds are manifested by prolonged inflammation, defective re-epithelialization and haphazard remodeling. Matrix metalloproteinases (MMPs) are zinc dependent enzymes that play cardinal functions in wound healing. Understanding the pathological events mediated by MMPs during wound healing may pave way in identifying novel drug targets for chronic wounds. Here, we discuss the functions and skewed regulation of different MMPs during infection and chronic tissue repair. This review also points out the potential of MMPs and their inhibitors as therapeutic agents in treating chronic wounds during distinct phases of the wound healing. This article is part of a Special Issue entitled: Proteolysis as a Regulatory Event in Pathophysiology edited by Stefan Rose-John.

1. Introduction

Wound healing is a cascade of partially overlapping events which proceed in an orderly fashion with precise functions of the cells involved. The process can be broadly classified into four different phases namely hemostasis, inflammation, proliferation and remodeling [1]. All the phases require functional coordination between different cell types like epidermal, dermal, endothelial and immune to initiate and culminate the tissue restoration process successfully. Tissue repair site is highly dynamic with several signaling pathways and cellular events that are imperative for executing multiple mechanisms such as blood clotting, fibroplasia, re-epithelialization, neovascularization, matrix deposition, etc. [1–5].

The skin being the largest organ of the body, plays an important role in fluid imbalance, thermoregulation and in protection against infection, harmful ultraviolet rays, etc. [6]. The integrity of the skin plays a pivotal role in maintaining physiological homeostasis and is of utmost importance for the viability of the inner tissues. Infliction to the epithelial layer during cutaneous wound breaches the integrity of the skin and compromises the mechanical barrier function that resist bacterial penetration. The multitude of bacteria in the external environment may now easily invade and interact with the sub-epithelium tissue structures including the extracellular matrix (ECM) and cause further damage [7]. If left untreated improper wound healing may cause infection and lead to life threating conditions. Therefore, proper, fast and complete healing of wounds is of high priority for the viability of internal organs and thus to the survival of the organism.

Matrix metalloproteinases (MMPs) are a group of zinc dependent proteases first discovered in tadpoles for its ability to degrade collagen fascicles in resorbing tail tissues of metamorphosing frogs [8]. Hitherto, 24 different MMPs have been identified in different tissues with varying substrate specificities and multiple functions [9,10]. In skin, MMPs are also known to regulate cell–cell and cell–matrix interactions through modulating and releasing cytokines, growth factors and other biological active fragments that are sequestered in the ECM [11–15]. MMPs modify cell surface receptors and junctional proteins, regulate processes including cell death and inflammation indirectly influencing cellular behavior [16,17]. MMPs play an important role during microbial infection, *in-vivo* studies on mice showed that degraded ECM products from different organs exhibit antimicrobial activity against wound pathogens adding another layer to MMPs' versatile functions [18]. On the other hand, in a chronic wound set up, the pathogen themselves secrete bacterial proteases that alter the proteolytic activity of the wound [19] and mediate degradation of structural matrix components [20,21]. There are several reports describing the general functions and implications of MMPs in normal wound repair process [22,23]. However there is a lacuna in the literature on the attributions of MMPs to the pathological state of chronic cutaneous wounds. In this review, we have precisely consolidated and discussed about the MMPs that are involved in chronic and infected non-healing wounds to explicitly highlight the

☆ This article is part of a Special Issue entitled: Proteolysis as a Regulatory Event in Pathophysiology edited by Stefan Rose-John.

* Corresponding author.
E-mail address: irit.sagi@weizmann.ac.il (I. Sagi).

[1] Equal contributions.

http://dx.doi.org/10.1016/j.bbamcr.2017.08.003
Received 5 May 2017; Received in revised form 1 August 2017; Accepted 4 August 2017
Available online 07 August 2017

importance of these proteases in dictating the healing trajectory.

2. Chronic wounds and infection

Unbalanced protease activity in conjunction with the exposed subepithelial and ECM structures promote bacterial colonization immediately after the wound formation. Growing evidences suggest that many strains of bacteria have an ability to specifically bind to matrix components which may facilitate their colonization and proliferation increasing the bacterial load in the damaged site [24,25]. The colonization of the wound by the invading bacteria is one of the hallmarks of chronic wound infections and poses a major problem in wound healing and treatment [26,27]. In fact, this phenomenon is so prevalent that it is suggested that virtually all open wounds are contaminated with microorganisms [28]. The type and quantity of these microorganisms vary from wound to wound and if the contamination involves a pathogenic bacterium then infection may occur [29]. Infection in contrast to contamination is an active disease that further delays the wound healing process [30]. Bacteria can also form communities in the wound and can promote the production of biofilm. Biofilm formation is a process that allows the bacteria to stick together in a self-produced matrix of extracellular polymeric substance [31]. The biofilm mesh enables the bacteria to evade many of the host immune system strategies to clear pathogens and is well known to delay wound healing [32]. Over 90% of infected chronic wounds with biofilm construct makes the elimination of bacteria harder and prolong healing [33]. It is also suggested that the type of pathogen persist in the wound can implicate on the fate of the healing [34]. Studies that aimed to identify the bacterial species that are present in chronic wounds showed that *Staphylococcus aureus* as one of the widely occuring bacteria followed by *Pseudomonas aeruginosa*, *Proteus mirabilis* and *Escherichia coli* [30,35].

3. Matrix metalloproteinases in infected and chronic wounds

Sustained proteolytic activity mainly due to augmented levels of MMPs, is one of the prime underlying factors responsible for the abnormal healing observed in chronic wounds [36,37]. The large family of MMPs has a collective ability to degrade almost all the ECM proteins and many non-ECM components like transmembrane proteins, cell surface receptors (e.g.: integrins), etc. [38–40]. A plethora of studies found that the endogenous tissue inhibitors of metalloproteinases (TIMPs) are also dysregulated in chronic wounds making the enzyme regulation awry [41–44]. The prolonged up-regulation of MMPs in the wound microenvironment not only cleaves several unintended targets, that includes growth factors and cytokines, but also largely tilt the ECM turnover impairing the repair process significantly [45–48]. The overview of different MMPs and their dysregulation in acute and chronic wound is schematically illustrated in Fig. 1

In normal physiological conditions the MMPs level and activity are stringently controlled by a myriad of factors [49]. But, in chronic wounds the expression of several MMPs are derailed both at mRNA and protein levels. The dysregulation is so apparently profound that a magnitude of 116-fold increase in the average protease activity was found in chronic wound exudates when compared to acute wound fluids [50]. The expression status of dysregulated MMPs in chronic wounds along with its role in tissue repair is listed in Table 1.

Infection with specific pathogen can modify ECM homeostasis differently, *S. aureus* infection leads to an increased levels of MMP-1 and -9 [51,52] whereas *P. aeruginosa* is well known to express elastase which can also impair wound healing [53]. Besides attracting host immune cells such as neutrophils and macrophages, which are primary sources of proteases, bacterial pathogens may further pull the balance of ECM towards degradation by secreting proteolytic enzymes that can activate host MMPs [54]. For example *P. aeruginosa* activates MMP-1, -8 and -9 by thermolysin protease [54]. Lipopolysaccharide associated serine proteinases activate pro-MMP-9 [55], while other bacterial proteases activate MMP-1, -3 and -9 [56,57]. In a study on chronic venous ulcers it was recorded that the levels of MMP-1 and -8 are specifically upholded in infected chronic wounds that are rich in *S. aureus*, *C. striatum* and *P. aeruginosa* when compared to high levels of MMP-2 and -9 in non-infected chronic wounds [58].

3.1. Collagenases

In general, chronic wounds exhibit a high collagenase activity with significantly increased levels of both MMP-1 and -8 along with diminished levels of their endogenous inhibitor, TIMP-1. MMP-1 also known as collagenase-1 has a wide range of substrate specificity and is capable of cleaving an array of ECM proteins and proteoglycans including collagen, aggrecan, versican, perlecan, etc. and hence directly influences the ECM deposition [62].

Wound healing studies on mice revealed that immediately after tissue insult the interaction of integrins on the keratinocytes with collagen-1 triggers MMP-1 expression which is vital for proper wound healing. The basal keratinocytes at the epithelial front secretes MMP-1 which cleaves the provisional matrix paving path for proper migration of the rapidly proliferating cells at the distal end. The levels of MMP-1 peaks at day one after the tissue insult and gradually decrease to basal level towards the completion of re-epithelialization. Certain laminin isoforms expression by keratinocytes during the final resolution phase acts as a downregulator of MMP-1 [63]. But, it seems in chronic wounds this tight regulation in the oscillation of MMP-1 levels is disrupted resulting in high concentrations of the enzyme leading to defective re-epithelialization. There are many reports evaluating the presence of excess amounts of MMP-1 in chronic wounds [64,65]. Muller et al. proved that the ratio of MMP-1/TIMP-1 is critical for tissue repair and in fact can be used as a predictor of the wound healing process [66]. *In vivo* investigations on lipodermatosclerosis, a skin induration preceding dermal ulcer, has also revealed that the expression of MMP-1 and -2 is increased in contrast to the decreased levels of TIMP-2 [67,68].

The immune response to an infection also contributes to the production of interstitial collagenases and gelatinases [58,69] and among the collagenases, neutrophil derived MMP-8 (collagenase-2) is directly involved in the pathogenesis of chronic non-healing wounds due to its overexpression and activation [44,70,71]. MMP-8 has stronger affinity towards collagen-1 which is one of the major interstitial collagens providing cellular signals and tensile strength necessarily required for a repairing tissue. In a 2-year long study, Amato et al. showed that MMP − 8 is significantly higher in non healing leg ulcers compared to healing ulcers [72]. But another contradicting report which came in the same year unraveled MMP-8 as a pro-healing enzyme [73].

Unlike MMP-1, the elevated expression of MMP-13 (collagenase-3) in the ulcer wound bed and its absence in the epidermis suggests that it has specific functions in granulation tissue formation and ECM remodeling. MMP-13, is also shown to have differences in spatial expression in non-healing wounds and is produced by distinct population of stromal cells [64]. Overall, these emerging studies indicate that collagenases are critical for ECM remodeling during wound healing which is a part of the tissue repair cycle.

3.2. Gelatinases

Gelatinases (A and B) are the predominant enzymes that are upregulated in the chronic wounds. Gelatinases have broader substrate preferences than collagenases and thus can degrade a wide gamut of ECM molecules [6,74]. Several reports indicate that gelatinases, MMP-2 (gelatinase A) and MMP-9 (gelatinase B) are found in higher amounts in chronic wound tissues and exudates when compared to acute wounds and adjacent normal skin [42]. Although these structurally similar enzymes can together cleave a large number of ECM molecules and hence inhibit important processes like angiogenesis, a high degree of substrate specificity is maintained between them. The elevated levels of