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MicroRNAs in diabetic wound healing: Pathophysiology and therapeutic opportunities ☆

Denizhan Ozdemir, MD. PhD[a], Mark W. Feinberg, MD[b,*]

[a] Department of Internal Medicine, Yale New Haven Hospital, Yale School of Medicine, New Haven, CT, United States
[b] Cardiovascular Division, Department of Medicine, Brigham and Women's Hospital, Harvard Medical School, Boston, MA 02115, United States

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ABSTRACT

Diabetic wound healing is an incompletely understood pathophysiological state. It comprises a range of potentially devastating and common complications of diabetes mellitus (DM) leading to intractable infections, lower extremity amputations, and associated cardiovascular morbidity and mortality. MicroRNAs (miRNAs) have emerged as important regulators in various physiological processes in health and disease through their ability to fine-tune cellular responses. Herein, we summarize the versatile roles of miRNAs implicated in diabetic wound healing in key stages including inflammation, angiogenesis, re-epithelialization, and remodeling. Furthermore, we highlight current evidence through which miRNAs exert control of gene expression and signaling pathways in the reparative response that may provide opportunities for therapeutic intervention for this potentially devastating disease state.

Introduction

Diabetes mellitus (DM) predisposes to several macro- and micro-vascular defects of end organs such as atherosclerosis, peripheral artery disease, retinopathy, nephropathy, neuropathy, and impaired wound healing [1]. One of the most debilitating causes of diabetes-associated mortality and morbidity stems from diabetic foot ulcers [2]. In particular, a non-healing diabetic foot ulcer represents a major risk factor for infection, foot deformity, or lower limb amputation [2]. Diabetes-related lower extremity amputation is the number one cause of non-traumatic limb amputations in the US than all other etiologies combined [3]. Of note, the 5-year mortality rate for patients with diabetic ulcers exceeds that of breast or prostate cancer, the most common forms of cancer in women and men, respectively [4]. Diabetic patients exhibit impaired reparative responses during the key stages of inflammation, angiogenesis, and re-epithelialization due to several contributing factors unique to diabetes [3,5,6].

Several pathophysiological features of impaired wound healing in diabetics are also hallmarks in other diabetic cardiovascular disease states [7]. For example, diabetic cardiomyopathy is characterized by dysfunctional ventricular remodeling independent of coronary artery disease, hypertension, and traditional cardiac risk factors [7]. Possible mechanisms leading to adverse ventricular remodeling associated with diabetes include metabolic disturbances, myocardial fibrosis, endothelial dysfunction and small vessel disease, and disruptions in metabolite utilization due to alteration in insulin signaling [8]. Albeit in different niches, heart and skin draw parallels in many aspects. In both diabetic tissues, capillary supply is compromised due to microangiopathy and abnormal endothelial responses [7]. There is also evidence of de-regulated composition of extracellular matrix and formation of reactive oxygen species [8]. Furthermore, the multi-cellular and tissue repair abnormalities in diabetic subjects may not be fully explained by the mere effect of glucose homeostasis [8].

Accumulating studies demonstrate that miRNAs play important roles for epigenetic control of physiological and pathophysiological wound healing [9,10]. In response to diabetic stimuli, the normal ability of miRNAs to fine-tune target genes can be drastically altered [10]. MiRNA expression can be detected extracellularly in the form of microvesicles or exosomes [11]. Herein, we present a summary of emerging roles of microRNAs in diabetic wound healing specifically focusing on their effects and underlying mechanisms in different cell types in a stage-specific manner. We highlight our current understanding of microRNA and target gene interactions that are perturbed in a range of cells (e.g., endothelial cells, fibroblasts, keratinocytes) important for conferring reparative properties. Lastly, we discuss how therapeutic intervention of microRNAs may provide an opportunity to overcome the natural progression of diabetic wound healing and its complications.

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* Corresponding author.
E-mail address: mfeinberg@bwh.harvard.edu (M.W. Feinberg).

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Brief primer on miRNA biology

MicroRNAs (miRNAs) are small noncoding RNA sequences averaging 22 nucleotides in length that can bind to the 3′-untranslated region (UTR) of target genes to modulate their expression through translational repression and/or mRNA degradation [12]. MiRNAs modulate a cluster of genes that are involved in numerous developmental and pathophysiological processes [13]. By targeting a given miRNA, it may provide amplified control in complex biological signaling pathways [13]. Depending on the degree of complementary sequence matching to the seed sequence of a miRNA in the 3′ UTR of a target mRNA, repression is mediated either by cleavage of mRNA if complete-alignment or suppression of translation if partial-alignment, or a combination of the two mechanisms [13]. A considerable number of miRNAs are evolutionarily conserved across species indicating that their fine-tuning of key targets has largely been preserved over time [13]. Finally, because miRNAs can be found in the circulation in extracellular vesicles, they can become potential harbingers of disease onset or progression by contemporary detection methodologies (e.g., real time polymerase chain reaction, RNA-Seq approaches, among others) [13].

MiRNAs governing stage-specific diabetic wound healing

The skin is the largest organ in the human body and consists of three main layers– the epidermis, dermis, and subcutaneous layers [14]. The outer layer, or epidermis, contains keratinocytes, dendritic cells, melanocytes, Langerhans cells, and sensory Merkel cells [14]. The inner layer, or dermis, provides the ground substance with elastic and collagenous fibers supplying nutrients with its vascular network [15]. A third layer, the hypodermis or subcutaneous layer, makes up the cushion layer with its adipocyte lobules [15]. As an outer barrier to external stress, skin constitutes the first layer of defense and constantly undergoes self-renewal [15]. In humans, perfect tissue healing has only been described in fetal skin, and quickly diminishes in the postnatal period [16]. Advanced age, nutritional or immunological deficiencies can be contributory comorbidities, but DM is the leading metabolic disease associated with defective wound healing [5].

Acute wound healing progresses through several overlapping stages: hemostasis, inflammation, angiogenesis, proliferation of granulation tissue and tissue deposition, and remodeling [17–19]. Failure to proceed in any of these phases leads to non-healing wounds [3]. A definitive role that miRNAs are involved in skin regenerative properties was identified through deficiency studies of key components of miRNA biogenesis [9]. For instance, the depletion of Dicer and DGCR8 genes impairs skin formation, proliferation of keratinocytes, stem cell activity, immune cell, and vasculature recruitment [9]. We dissect the role of microRNAs and their targets in each phase impacted by DM below.

Hemostasis

Immediately after the skin barrier is breached, coagulation is activated through the formation of a fibrin clot via intrinsic and extrinsic coagulation cascades [20]. Homeostasis is achieved within minutes in order to stop the blood loss and pave the way for influx of different cell types [20]. The blood clot assures a provisional matrix architecture that releases growth factors and cytokines to mobilize immune cells into the injury site [17]. Among the cytokines released into the injured tissue are PDGF (platelet-derived growth factor), EGF (epidermal growth factor), and TGF-β (transforming growth factor-β) [21]. While miRNAs governing this early phase of diabetic wound healing has not been specifically defined, several miRNAs implicated in thrombus formation may be contributory (Reviewed in [22]).

Inflammation

Injury is one of the most potent stimuli for the release of inflammatory cytokines [23]. Timing of wound-induced inflammation is a well-regulated process that takes place between 1–4 days after the initial insult [17]. During this period, the goal of the immune system is to eliminate any invading pathogens that can damage tissues [17]. After the infecting agents have been eradicated, inflammation has to be discontinued in order for repair to proceed. Anti-inflammatory mechanisms set off the resolution of inflammation and progression of a reparative cascade [24]. The failure to progress is one of the root causes for impaired diabetic wound healing [5]. Epidermal keratinocytes contribute to the inflammatory phase via rapidly activating Toll-like receptors (TLR) 1–6 and 9 [15,25]. Infiltrating neutrophils release pro-inflammatory cytokines that, in turn, induce the recruitment of monocytes to the wound [17]. Imported monocytes can differentiate into a pro-inflammatory M1 phenotype in the presence of pro-inflammatory cytokines [26]. Studies have shown that an abundant state of pro-inflammatory infiltrates with macrophages and neutrophils is present in chronic ulcers, which can lead to enhanced levels of matrix metalloproteinases (MMPs) [27]. Consistent with this, anti-inflammatory genes such as Ym1 and Arg-1, are reduced in wounds of db/db mice [27]. The net effect is excessive degradation of extracellular matrix (ECM) and inadequate migration.

The second phase of inflammation attracts fibroblasts and myofibroblasts to the injury site and initiates the formation of ECM reconstitution [17]. TGF-β, a cytokine family important in development and repair, acts as a chemoattractant for infiltrating cells which in turn further enhances levels of TGF-β via a feed-forward loop [28,29]. Among other mediators, eicosanoids are comprised of a family of metabolites including prostaglandins, thromboxanes, prostacyclin, leukotrienes, and lipoxins [30]. After a critical substrate amasses for rebuilding the injured tissue, inflammation is gradually extinguished by a combination of anti-inflammatory cytokines and M2 macrophages [26]. Recent studies of diabetic wounds indicate that a failure to suppress the subsequent competition between pro- and anti-inflammatory signals lead to a misbalanced environment for healing [3,5,25]. It prevents the transition to a proliferative phase and reconstitution of the ECM [27,31]. Continual exposure to external pathogens increases the bacterial load in wounds and sustains the influx of inflammatory cells [5].

Several microRNAs have been implicated in regulating the inflammatory phases of diabetic wound healing. For example, Xu et al. demonstrated that miR-146a expression was significantly reduced in the wounds of diabetic mice compared to controls [32,33]. Further studies verified that miR-146a targets pro-inflammatory mediators such as IRAK1 and TRAF6. Correction of miR-146a expression via repletion of mesenchymal stem cells (MSC) led to enhanced diabetic wound closure suggesting that dynamic expression of miR-146a may actively control the inflammatory state in diabetic wounds. Ramirez et al. studied *Staphylococcus aureus*, a frequent colonizer, in diabetic human and porcine wounds [34]. *S. aureus* induced miR-15b-5p expression at the wound site leading to impaired DNA repair by repressing downstream targets such as IKBKB, WEE1, FGF2, RAD50, MSH2, and KIT, which in turn sustained a pro-inflammatory state. Li et al. studied the role of miR-132 in db/db mice and patients with DM compared to the healthy controls [35,36]. MiR-132 expression was reduced in diabetes, and gene ontology analyses of miR-132 showed an association with inflammatory pathways including NF-κB, NOD-like receptor, TLR, and TNF signaling pathways. The topical application of liposomal-mediated miR-132 mimic delivery in wounds led to

accelerated skin healing in mice and re-epithelialization of human *ex vivo* skin wounds via inhibition of pro-inflammatory cytokines in macrophages, monocytes, and keratinocytes. Additionally, miR-132 may facilitate the transition from the inflammatory to the proliferative phase by targeting Hb-EGF [35].

Other miRNAs implicated in regulating inflammation and wound healing include miR-155, miR-191, and miR-200. Previous studies showed that inhibition of miR-155 can dampen inflammation [37,38]. Ye et al. examined the inhibition of miR-155 in the setting of streptozotocin-induced diabetes in Sprague-Dawley rats [39]. Inhibition of miR-155 was associated with reduced MPO-positive cells and increased numbers of angiogenic markers suggestive of ECM build-up such as collagen 1, TGF-β1, and α-SMA, and improved diabetic wound healing. Investigating the paracrine effects of cells in the wound site, Ti et al. extracted exosomes containing let-7b from LPS-primed mesenchymal stromal cells [40]. Local administration of exosomes into the wound site of streptozotocin-induced diabetic rats decreased inflammation through a TLR4/NF-κB/STAT3/AKT signaling pathway that regulates macrophage plasticity towards a M2 profile. In another study, miRNA plasma profiling was performed in an effort to distinguish between the levels of different miRNAs in patients with DM-associated peripheral artery disease (PAD) and chronic wounds compared to patients with DM without these conditions. While miR-191 and miR-200b were decreased in DM subjects compared to healthy controls, the presence of PAD alone with DM did not affect plasma expression levels of these miRNAs. In contrast, miR-191 and miR-200b expression were increased in the presence of DM with PAD and chronic wounds. In addition, both miRNAs were positively correlated with higher levels of inflammation-associated markers such as C-reactive protein (CRP) [41,42]. Overexpression of miR-191 reduced tube formation capacity, migration, and zona occludens-1 expression in human dermal endothelial cells. The authors hypothesize that in response to inflammatory stress, endothelial cells secrete miR-191 into blood, which may be taken up by distant fibroblasts or microvascular endothelial cells of inflamed skin wounds. Because miR-191 targets ZO-1, recipient cells suppress angiogenesis or cell migration leading to delayed wound healing in subjects with DM and chronic wounds.

Angiogenesis

The level of tissue oxygenation is one of the initial microenvironmental cues that activate new blood vessel formation [43]. As the injured tissues are cleared, the progression of healing relies upon a new supply of oxygen and nutrients [43]. Angiogenesis is the main process during wound healing where sprouting of new endothelial cells occurs from pre-existing vessels [44]. In contrast, vasculogenesis consists of migration of bone marrow-derived progenitors [43]. An array of different cell types including fibroblasts, platelets and macrophages stimulate endothelial cell migration via secretion of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), tumor necrosis factor (TNF-α), and platelet derived growth factor (PDGF) [45]. The study of hypoxia-responsive genes led to the discovery of HIF-1 (hypoxia-inducible factor-1) transcription factors [46]. HIF-1 and associated factors are rapidly up-regulated in tissues where vascular supply is disrupted [47]. VEGF is the most robust regulator of nitric oxide that up-regulates VEGF in a feed-forward loop [48]. Collectively, VEGF and NO increase EC migration, decrease adhesion, and vasodilate organizing vascular networks [49]. Optimization of angiogenesis remains one of the most crucial parameters in wound healing [17]. Deprivation of angiogenic factors in diabetic wounds hinders the skin's ability to launch a proper response to angiogenic stimuli [49]. In addition, a hostile diabetic wound environment promotes dysregulation of key signaling pathways such as those associated with VEGF, epidermal growth factor, bone morphogenetic protein (BMP), and TGF-β [29].

A growing list of miRNAs have been implicated in controlling diabetic wound angiogenesis. For example, our previous studies showed that miR-26a could suppress endothelial cell proliferation, migration, and network tube formation by targeting SMAD1, a downstream regulator of the BMP pro-angiogenic signaling pathway [50]. In addition, miR-26a is robustly increased in diabetic wounds *in vivo* or in response to high glucose in endothelial cells *in vitro* [51]. Neutralization of miR-26a with local intradermal anti-miRs effectively suppressed miR-26a expression, de-repressed its target SMAD1, and promoted wound edge angiogenesis, granulation tissue thickness, and diabetic wound closure rates *in vivo*. Another miRNA, miR-27b, has been shown to augment the function of bone marrow-derived angiogenic cells (BMAC), including proliferation, adhesion, tube formation, and delayed apoptosis [52,53]. Diabetic db/db mice had reduced expression of miR-27b, and replacement of miR-27b reduced expression of thrombospondin-1 (TSP-1), semaphorin 6A expression, and p66shc, an effect that improved topical cell therapy of diabetic BMACs on diabetic skin wound closure and angiogenesis [52,53].

Other miRNAs implicated in angiogenesis and diabetic wound healing include miR-15b, miR-200, and miR-205b. MiR-15b may regulate proangiogenic progenitor stem cells to influence diabetic wound healing. MiR-15b is induced in diabetic wounds in mice compared to non-diabetic controls. Treatment with MSCs led to improved diabetic wound healing, a significant decrease in miR-15b expression levels, and up-regulation of pro-angiogenic genes [54,55]. A recent study demonstrated that the activity of inositol-requiring enzyme 1 (IRE1a), an endoribonuclease important in the endoplasmic reticulum-associated stress signals, is diminished in DM [56]. Notably, IRE1α RNase attenuated the maturation of two pre-miRs, pre-miR-466 and pre-miR-200 [57]. In mature form, increased activity of miR-200 and miR-466 led to suppression of pro-angiogenic angiopoietin 1 (ANGPT1) and decreased angiogenesis. Pizzino et al. utilized a combination of miR-15b and miR-200 inhibitors, both of which activate the VEGF pathway, to study the effects on diabetic wound healing [55]. Inhibition of these anti-angiogenic miRs led to improved wound healing in diabetic mice. Chan et al. showed that the inflammatory cytokine TNF-α can induce miR-200b expression, which produces angiostatic effects by targeting globin transcription factor binding protein 2 (GATA2) and vascular endothelial growth factor receptor 2 (VEGFR2) in diabetic mice [58]. MiR-200b overexpression attenuated cellular motility, proliferation, angiogenesis, and stemness at the wound edges. These detrimental effects were reversed using anti-miR-200b or overexpressing GATA2 and VEGFR2 *in vivo*. In another study, miR-92a expression was elevated in chronic wounds as compared to acute wounds in both human and db/db mice [59]. An approach utilizing an innovative light-inducible anti-miR-92a injected into the skin was undertaken to avoid systemic toxicity [59]. Irradiation-activated intradermal delivery of anti-miR-92a did not change miR-92a expression in other organs. The upregulation of miR-92a targets, Itga5 and Sirt1, led to augmented endothelial cell proliferation, angiogenesis, and wound closure in db/db mice. These findings highlight the possibility of how light can be used to activate local anti-miRs *in vivo*.

Finally, miRNAs can regulate the function of non-endothelial cell types in diabetic wounds to control angiogenesis. For example, miR-205-5p targets VEGF at the protein translation level in MSCs [60]. Zhu et al. showed that inhibition of miR-205-5p in MSCs of diabetic NOD/SCID mice improved the healing of diabetic foot ulcers, potentially through augmentation of VEGF-mediated angiogenesis [60]. Using a different approach, Tao et al. overexpressed miR-126-3p in the form of exosomes, named SMSC-126-Exos, from synovial MSCs [61]. Later, miR-126-containing exosomes were

Table 1
MicroRNAs and their targets involved in diabetic wound healing.

MicroRNAs	Mechanism of Action	References
Stage: Inflammation		
Pro-inflammatory		
miR-15b-5p	Suppresses DNA repair genes such as IKBKB, WEE1, FGF2, RAD50, MSH2, and KIT leading to excess accumulation of pro-inflammatory double stranded breaks. Induction of miR-15b-5p inhibits healing in human and porcine diabetic wound models	[34]
miR-155	Activates MPO-positive neutrophils and macrophages. Inhibition of miR-155 improves wound healing in diabetic rats	[37,38,39]
miR-191 and miR-200	Associated with increased CRP and pro-inflammatory cytokines. Predicts more aggressive diabetes-related wounds	[41]
Anti-inflammatory		
Let-7b	Suppresses TLR4/NFκB/STAT3/AKT cascade. Overexpression augmented wound healing in diabetic rats	[40]
miR-132	Decreases NF-κB, NOD-like receptor, toll-like receptor, and tumor necrosis factor signaling and regulates the transition from inflammatory to a proliferative phase by targeting HB-EGF. MiR-132 blockade impaired healing in db/db mice and in healthy ex vivo skin wounds	[35,36]
miR-146a	Negatively regulates the inflammatory response (IL-8 and RANTES) by targeting IRAK1 and TRAF6. Correction of miR-146a levels improves wound healing in diabetic mice	[32,33]
Stage: Angiogenesis		
Pro-angiogenic		
miR-27b	Overexpression rescues impaired angiogenic cell function via TSP-1 suppression and improves wound healing in diabetic mice	[52,53]
miR–126–3p	Promotes angiogenesis, endothelial cell proliferation, and migration through Akt and ERK1/2. Overexpression improves wound healing in diabetic mice	[61]
Anti-angiogenic		
miR-15b	Suppresses Akt3, VEGF, and (HIF)−1α. Inhibition of miR-15b improves wound healing in diabetic mice	[54,55]
miR-26a	Inhibits angiogenesis by targeting BMP1/SMAD1 signaling; Neutralization of miR-26a promotes angiogenesis and wound healing in diabetic mice	[50,51]
miR-92a	Suppresses ITGA5 and ITGB5. Neutralization of miR-92a promotes angiogenesis and wound healing in diabetic mice	[59]
miR-200/466 families	Represses ANGPT1 mRNA translation. Please see the effect of miR-200b inhibition below	[57]
miR-200b	Suppresses angiogenesis by targeting ETS1, GATA2, and VEGFR2. Inhibition of miR-200b improves wound healing in diabetic mice	[55,58]
miR–205–5p	Suppresses translation of VEGF mRNA. Inhibition of miR–205–5p augmented wound healing in diabetic mice	[60]
Stage: Re-epithelialization and Remodeling		
miR-21	Promotes fibroblast migration and re-epithelialization through TGF-β1-inducible expression. Expression of miR-21 is decreased during diabetic wound healing	[63,69]
miR-25	Associated with decreased expression of TGF-β1, SMAD-3, Col1α1, and Col3α1. Expression of miR-25 is increased in the wounds of diabetic mice	[71]
miR-29a	Silences TGF-β by targeting TAB-1. Expression of miR-29a is increased in the wounds of diabetic mice	[71]
miR-29b	Shows inverse expression with fibronectin, collagen type I, collagen type III, and MMP9. De-repression of miR-29b reduction led to improved wound healing in diabetic mice	[31]
miR-191	Modulates cellular migration via paracrine regulation of zonula occludens-1 to delay wound healing. Associated with more aggressive diabetes-related PAD and wounds	[41,42]
miR-198	Inhibits keratinocyte migration and proliferation by suppressing DIAPH1, PLAU, and LAMC2. Induction of the mature miRNA form naturally eliminates the possibility of FSLT1 mRNA transcription due to origination from a common single strand. Failure of this switch occurs in chronic diabetic ulcers in humans	[68]

incorporated into chitosan dressings. This particular system stimulated the proliferation of human dermal fibroblasts and human dermal microvascular endothelial cells *in vitro* through Akt and ERK1/2 signaling. *In vivo* testing in the skin of diabetic rats showed improved wound healing through augmented re-epithelialization, angiogenesis, and collagen maturity. Finally, Liang et al. described the differential expression of microRNAs in primary fibroblasts in diabetic foot ulcers compared to non-diabetic foot ulcers [62]. The study found three microRNAs (miR-34a-5p, miR-21-5p, and miR-145-5p) were increased and their predicted target gene networks including CD47, RECK, IRS1, and PDGFRA were reduced. However, the functional roles of these miRNAs were not defined. Madhyashta et al. also identified that miR-21 is increased in diabetic skin but decreased during diabetic wound healing. Overexpression of miR-21 enhanced fibroblast migration *in vitro* [63]. Further studies will be needed to clarify its role *in vivo*.

Re-epithelialization (proliferation)

The integument is a dynamic organ with versatile functions such as guarding the body from external pathogens, preventing the loss of fluids, and unceasingly replenishing the exfoliation of stratified epithelium [15]. Keratinocytes, a major cellular player of the epidermis, are pivotal in these processes by participating in migration, proliferation and differentiation [64]. However, keratinocytes not only serve in maintenance functions but also in restoring the intact skin through re-epithelialization [18]. Without an epithelial barrier fully covering the denuded tissue, a wound is at risk of creating a portal for infection [18]. In response to skin injury, re-epithelialization commences as early as a few hours [17].

Strikingly, the properties of keratinocytes in chronic diabetic wounds show remarkable differences than acute wounds or intact skin [65]. The term parakeratosis, a hyperproliferative state of stratified epithelium throughout all 5 layers, is the defining characteristics of chronic wounds by c-myc activation [65]. Collectively, several cytokines and growth factors modulate wound healing events and the delicate balance of overlapping progression through multiple phases of healing [66]. However, imbalances in DM in growth factors, and cellular and structural components can stall the advancement of process [5]. Several studies compared the expression pattern of miRNAs in epidermal lineage and wound healing [67]. Examples of specific miRNAs and their actions can be found in Table 1. For example, miR-198 lies within a protein-coding

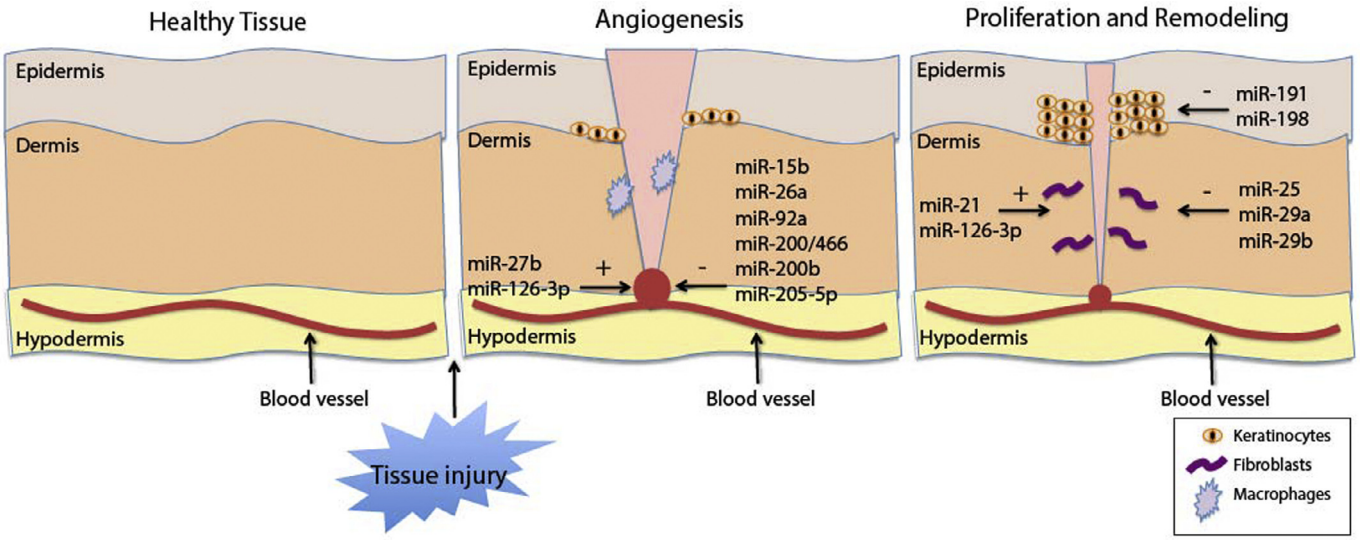

Fig. 1. Summary of stage-specific microRNAs in diabetic wound healing. In response to tissue injury in diabetic wounds, there is an altered balance of endothelial microRNAs (miRs) that negatively regulate the induction of angiogenesis. Adverse cellular proliferation and tissue remodeling ensues due to maladaptive expression or function of miRs in dermal, myofibroblast, or epithelial cell types. Targeting specific miRs may overcome stage-specific defects in diabetic wounds and promote tissue repair. (–) and (+) indicates negative or positive regulation, respectively.

gene named follistatin-like 1 (FSTL1) in the human genome [68]. Human *ex vivo* skin culture studies demonstrated a dynamic inverse interplay of miR-198 and FSTL1 expressions. At steady state, miR-198 prevails in healthy skin tissues whereas FSTL1 mRNA and protein are absent. Upon wounding, miR-198 is rapidly reduced while FSTL1 expression soars, culminating in keratinocyte migration. The disruption of this molecular switch is illustrated in non-healing diabetic ulcers where miR-198 maintains high levels of expression and corresponding deficiency of FSTL1. Interestingly, the mechanics of this precise regulation, dubbed as "see-saw expression" by the authors, originates from a single transcript by which it can only be processed to either miR-198 or FSTL1 rather than complementary binding of miR-mRNA. If the single transcript is committed to the formation of miR-198, the mature miRNA exerts its effect by targeting DIAPH1, PLAU, and LAMC2. Finally, Geiger et al. investigated the "miR-acrine" effects of human fibroblast-derived exosomes in which matrix-promoting (miR-21), anti-inflammatory (miR124a, miR-125b), and pro-angiogenic (miR-126, miR-130a, miR-132) properties were enriched after stimulation with platelet-derived growth factor, fibroblast growth factor-2, and TGF-β1 [69]. Indeed, this particular approach also avoided potential immunogenicity of fibroblasts without impairing their pro-healing effects. Local injection of this human exosome concentrate accelerated wound closure in the skin of diabetic db/db mice.

Remodeling (maturation) phase

In healthy wounds, the longest phase of healing consists of the remodeling phase, which can take several weeks to months. The interim matrix scaffold formed during prior phases is substituted by granulation tissue made up of fibroblasts, collagen fibers, and a vascular supply network [27,28]. The main characteristic of this phase is the deposition of mature collagen in an orderly manner [28]. In order to achieve permanent tissue deposition, a temporary scaffold tissue needs to be turned over by MMPs [27]. Often, the reconstitution and breakdown of tissue occurs simultaneously [19]. Initially, the granulation tissue is mainly composed of fibrin and fibronectin which are products of macrophages [19]. Next, glycosaminoglycans and proteoglycans are secreted by fibroblasts [19]. The temporary matrix provides a preliminary scaffold. With the help of MMPs such as collagenase and gelatinases, the wound is replaced by a more durable and organized matrix made of collagen type I converted from initially-formed collagen type III [70]. TGF-β contributes to myofibroblast differentiation allowing for wound contraction and filling-in defects [29]. As the tissue reverts back to its original architecture, the number of immune cells and blood vessels decrease [70]. In diabetic wounds, several reparative processes are afflicted [3]. For instance, although keratinocytes are hyperproliferating, their migration is diminished [3]. The fine balance between formation and digestion of tissue constituents are favored for increased breakdown as represented by an increase in activated MMPs [27]. Angiogenesis is also adversely diminished in the reparative phase of diabetic wounds [5]. Finally, altered procollagen expression and orientation of alpha-smooth muscle actin (α-SMA) staining in fibroblasts are observed in a diabetic mice, resulting in increased matrix rigidity and reduced contractility of the diabetic wounds [39]. Interestingly, TGF-β is repressed in diabetic and chronic wounds, an effect that may contribute to a myriad of molecular abnormalities in diabetic ulcers [29,71].

An emerging handful of miRNAs have been implicated in the remodeling phase. Xu et al. explored the inhibitory effect of MSCs on modulating MMP9 activity in diabetic mice [31]. When proteolytic activity of MMP9 is elevated, miR-29b expression is reduced in the wounds of diabetic db/db mice compared to non-diabetic mice. Treating the wounds with MSCs attenuated MMP9 activity and de-repressed miR-29b levels. Although this study did not demonstrate a direct causal relationship of miR-29b and MMP9 activity, they may be helpful as surrogate markers with a high degree of correlation by linear regression analysis. Another study investigated collagen synthesis in diabetic wounds compared to non-diabetic in mice by inducing recurrent injuries (one week apart for two weeks). This injury approach decreased expression of pro-matrix producing gene markers including *TGF-β1, SMAD3, Col1α1*, and *Col3α1 expression* in diabetic wounds [71]. Interestingly, all time points displayed diminished collagen production with the exception of last measurement. This time point correlated with increased expression of collagen pathway genes and a concomitant decrease in miR-25 and miR-29a, a finding temporally related to delayed wound healing.

Conclusions

Diabetes poses an independent risk factor for cardiovascular disease as well as normal wound healing [72]. The impact of diabetes on wound healing is mediated through cellular disturbances for individual cell types including endothelial cells, immune cells, fibroblasts, which may shed light on the challenges faced in other cardiovascular disease states associated with adverse tissue remodeling such as diabetic cardiomyopathy, post-myocardial infarction, or critical limb ischemia, among others [72]. For some of the well-documented common metabolic abnormalities such as endothelial dysfunction, there are no current targeted therapies. Therefore, despite optimal medical therapy, management of diabetic wound healing remains relatively stagnant. Complex pathophysiology of diabetes impairs cellular and molecular responses, and the expression of miRNAs is no exception. Multiple studies demonstrate differential expression of miRNAs in diabetic wounds compared to non-diabetic wounds. Furthermore, miRNA expression may be utilized in combination with other biomarkers to inform potential maladaptive or delayed wound healing in diabetics. Emerging studies on miRNA expression and function have uncovered new information on signaling pathways, molecular interactions, and gene networks. Collectively, miRNAs serve to fine-tune pathophysiological responses in each of the four major phases of diabetic wound healing (Fig. 1). As such, manipulation of stage-specific miRNAs may provide opportunities for therapeutic intervention. Given the diverse targets of these stage-specific miRNAs, it will be of future interest to explore whether targeting a combination of miRNAs may provide a more effective means to achieve pro-healing properties. Future investigation on the role of miRNAs using innovative gain- and loss-of-function studies by local intradermal delivery in both diabetic mouse models of wound healing and in human skin organoid preparations may help to close the pre-clinical gap in translating these findings closer to the clinic.

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