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Critical Review

New Insights into MicroRNAs in Skin Wound Healing

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Abstract

Chronic wounds are a major burden to overall healthcare cost and patient morbidity. Chronic wounds affect a large portion of the US, and billions of healthcare dollars are spent in their treatment and management. microRNAs (miRNAs) are small, noncoding double-stranded RNAs that post-transcriptionally downregulate the expression of protein-coding genes. Studies have identified miRNAs involved in all three phases of wound healing including inflammation, proliferation, and remodeling. Some miRNAs have been demonstrated *in vitro* with primary keratinocyte wound healing model and *in vivo* with mouse wound healing model through regulation of miRNA expression to affect the wound healing process. This review updates the current miRNAs involved in wound healing and discusses the future therapeutic implications and research directions. © 2015 IUBMB Life, 67(12):889–896, 2015

Keywords: *microRNAs; wound healing; skin; pathogenesis; drug discovery; keratinocytes*

Introduction

Chronic wounds are a significant burden to a large portion of the population and in the hospital setting, affecting the overall morbidity and mortality of patients. It is estimated that chronic wounds affect approximately 6.5 million patients in the United States (1). Moreover, approximately $25 billion is spent annually in the US toward the treatment of chronic wounds (2). Patients at risk for delayed wound healing include diabetics, immunocompromised, elderly, and those with impaired venous and arterial circulation (3). Wound healing involves three major stages: inflammation, proliferation, and remodeling (4). Chronic wounds remain locked in the inflammatory stage (5). Understanding the key regulators of wound healing, including recently discovered microRNAs (miRNAs), may identify target therapies to prevent chronic nonhealing wounds.

The majority of our genome consists of nonprotein-coding DNA. For decades, these regions were considered insignificant, but the discovery of miRNAs in 1993 changed this paradigm. miRNAs are nonprotein-coding small endogenous RNAs that are approximately 21–25 nucleotides in length and are believed to regulate up to 50% of all protein-coding genes (6). Their roles are to post-transcriptionally control the expression

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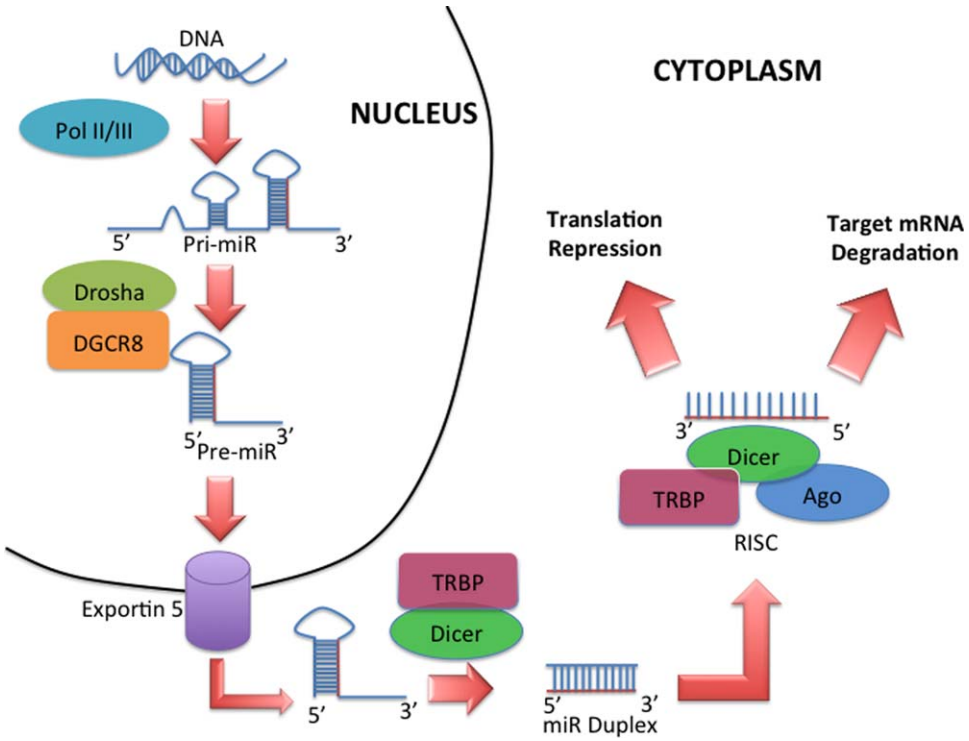

FIG 1

MiRNA biogenesis pathway. In the nucleus, RNA polymerase II-III transcribes miRNA genes, generating long primary transcripts (pri-miRNAs), and the RNase III-type enzyme Drosha further processes the pri-miRNA, yielding a hairpin precursor miRNA (pre-miRNA). Exportin 5 transports the pre-miRNA from the nucleus into the cytoplasm. The pre-miRNA hairpins are further processed into unstable miRNA duplex by Dicer. One strand, mature miRNA, in the duplex is incorporated into Ago protein and forms a complex, the RISC together with Dicer. Once incorporated into a RISC, the mature miRNA regulates the target genes by destroying the mRNA through direct cleavage or by inhibiting protein synthesis through binding to 3′ UTR of targeting gene.

of protein-coding genes by binding to messenger RNA (mRNA), leading to degradation or suppression of translation (7,8). miRNAs have been extensively studied in all fields of medicine. Notably although, the discovery of miRNA influence on wound healing may change treatment and management of chronic wounds in the future. The dysregulation of miRNAs may be a contributing factor to nonhealing wounds. Identification of miRNAs involved in each phase of wound healing will further expose potential therapeutic pathways. We here present a comprehensive review of the current studies on miRNAs in wound healing along with their implications in wound healing management.

miRNA Biogenesis

miRNA biogenesis involves several steps (Fig. 1). miRNAs are first transcribed from DNA segments by RNA polymerase II or III and capped at the 5′ end with a polyA tail, forming a pri-miRNA (9). Nuclear RNaseIII-type enzyme Drosha (RNASEN) and DGCR8 (DiGeorge Syndrome Critical Region 8) cleave this complex into a 60–70 nucleotide long pre-miRNA with a typical stem loop structure (10). The pre-miRNA is then actively transported out of the nucleus *via* Exportin-5 (XPO5) (11). Once the precursor is in the cytoplasm, RNaseIII-type enzyme Dicer cuts the hairpin loop that forms the double stranded miRNA, approximately 21–25 nucleotides in length. One strand of the miRNA is incorporated into RISC, an RNA-induced silencing complex (12). The newly formed miRNA–RISC complex works to stop mRNA translation *via* imperfect base pairing at the 3′ untranslated (UTR) region (13). This leads to regulation of target genes *via* degradation of mRNA or inhibition of protein translation (14).

miRNA in Wound Healing

miRNAs are present in all types of tissue and regulate a wide variety of processes at the cellular level including proliferation, differentiation, and apoptosis (15). Normal skin development is dependent on a high manifestation of miRNAs and Dicer enzyme within the epidermis and hair follicles (16).

Skin serves as a primary protective barrier against the outside environment as well as a form of UV defense, thermoregulation, pigmentation, and means of reducing water loss (17). Once this barrier is disrupted, wound healing begins. Wound healing classically involves three phases: inflammation, proliferation, and remodeling (4). miRNAs act as both agonists

and antagonists in the process of restoring barrier function of the skin. Changes in the expression of specific miRNAs during different phases can be associated with abnormal wound healing (17). With greater understanding of the roles of miRNAs in each phase of wound healing, we can appreciate how their dysregulation leads to delayed or impaired wound restoration. Notably, miRNAs are potentially involved in wound healing at virtually every step in a highly coordinated manner (Table 1).

Roles of miRNAs in the Inflammatory Phase

The first phase of wound healing, inflammation, begins immediately after the skin barrier is disrupted and the clotting cascade is activated. As hemostasis occurs through the establishment of a fibrin clot, chemokines and cytokines such as platelet derived growth factor (PDGF), interleukin-1 (IL-1), transforming growth factor-β (TGF-β), and tumor necrosis factor-α (TNF-α) are released into the bloodstream. Due to the breach of the protective barrier, the underlying tissue becomes vulnerable to pathogens and the risk of infection increases. Immune cells such as monocytes and neutrophils are triggered to travel to the wound *via* the aforementioned cytokine and chemokine signaling (55,56). This signaling, along with receptor gene expression, is just one of many avenues in which miRNAs regulate the inflammatory phase.miR-146a investigation has shown that it is extensively involved as a negative regulator of inflammation, specifically in the innate immune system (57). Macrophages are regulated by miR-146a and miR-155, which promote production of cytokines and growth factors necessary for monocyte differentiation into macrophages (19,24). Toll-like receptor-4 (TLR-4)-mediated inflammation is regulated by miR-21's effects on programmed cell-death protein 4 (PDCD4) expression (21). Interestingly, miR-146a, miR-155, and miR-21 are reported to be linked to wound healing processes (27,58). While miRNAs certainly promote and induce inflammation, they also work to downregulate and terminate the phase once necessary (24). Prolongation of the inflammatory phase can lead to tissue damage and prevent proper wound healing, leading to chronic wounds (59). As the inflammatory phase comes to an end through the reduction in number of macrophages and neutrophils, the proliferation phase begins.

Roles of miRNAs in the Proliferation Phase

The proliferation phase commences 2–3 days after injury occurs and includes the recruitment of fibroblasts to the wound site to initiate the formation of granulation tissue, deposition of collagen, glycosaminoglycans, keratinocyte differentiation, epithelialization, and wound contraction (59,60). Fibroblasts become the main cell type in the wound by the end of the first week (4). Cytokines have variable effects on the miRNAs in fibroblasts, such as miR-155, which increases fibroblast migration (29). Fibroblasts excrete collagen and fibronectin to form a new extracellular matrix (ECM), the foundation of granulation tissue (59). Moreover, miRNAs such as miR-21, -99, -155, -184, -198, -203, -205, -210, and -483-3p work to regulate the production, differentiation, and migration of keratinocytes (27,29–32,34,40).

Within the proliferation phase, new blood vessels begin to form to readily supply the healing area with plentiful oxygen and nutrients *via* angiogenesis/neovascularization. This process is vital to fueling the activity of fibroblasts and epithelial cells. The hypoxic environment stimulates hypoxia-inducible factor (HIF) to activate genes including vascular endothelial growth factor (VEGF) and glucose transporter 1 (GLUT1) to boost angiogenesis (60,61). Hypoxia-sensitive miRNAs including miR-23, -24, -26, -27, -103, -107, -181, -210, and -213 are transcriptionally controlled by HIF (62). During this phase, the tissue appears erythematous due to the newly acquired capillary network. Multiple miRNAs are involved in proangiogenic and antiangiogenic regulation including miR-15b, -16, -17-92, -126, -130a, -210, -221, -222 -296, -320, -378, and -503 (35–41,43–50,54).

In addition, keratinocytes migrate from the wound edge to the wound site and begin to proliferate and differentiate to restore skin integrity—a process that can be inhibited by various miRNAs including miR-198, -203, and -483-3p (31,32,34). Re-epithelialization and wound contraction then occur as new epithelial cells migrate over the wound and myofibroblasts assist in decreasing the wound magnitude through tightening. Each cell undergoes the process of apoptosis when its purpose is fulfilled.

Roles of miRNAs in the Remodeling Phase

When the wound is closed, the remodeling phase begins. This phase involves adjustment of the ECM, collagen remodeling to type I from type III collagen, and scar formation in place of the temporary granulation tissue. Realignment of the tissue along tension lines occurs, allowing for ideal wound healing. During this phase, blood vessels to the wound decrease and cellular activity begins to quiet in preparation for completion. In specific, miR-29a regulates dermal fibroblasts by controlling their contractility through targeting TABL1 (51). miR-192/215 increases the expression of E-cadherin by repressed translation of ZEB2 (54), while E-cadherin plays a role in re-establishing barrier integrity of skin. Of all the phases of wound healing, the remodeling phase still requires further investigation into discovering the various miRNAs involved in regulation.

miRNAs Regulate *in vitro* Wound Healing Processes

Through the use of *in vitro* primary human keratinocyte models, studies have demonstrated the roles of miRNAs in

TABLE 1 *miRNAs in wound healing*

miRNA	*Target*	*References*
Inflammation		
Proinflammatory		
miR-140	PDGF receptor	(18)
miR-155	SOCS1	(19)
Anti-inflammatory		
miR-16	COX2	(20)
miR-21	PDCD4	(21)
miR-105	TLR2	(22)
miR-125b	TNF-α	(23)
miR-146a,b	TRAF6, IRAK1, STAT1	(24)
miR-203	TNF-α, IL24	(25)
miR-223	Mef2c	(26)
Proliferation		
miR-21	TIMP3, TIAM1	(27)
miR-99	IGF1R, mTOR, AKT1	(28)
miR-155	KGF, FGF-7	(29)
miR-184	Akt	(30)
miR-198	DIAPH1, PLAU, LAMC2	(31)
miR-203	RAN, RAPH1	(32)
miR-205	SHIP2, Rho-ROCK1	(30)
miR-210	E2F3, ISCU 1/2	(33)
miR-483-3p	MK2, MKI67, YAP1	(34)
Pro-angiogenic		
miR-17-92	TSP-1	(35,36)
miR-126	Spred1, PIK3R2	(37,38)
miR-130a	GAX, HOXA5	(39)
miR-210	EFNA3 (ephrin-A3)	(40)
miR-296	HGS	(41)
miR-378	Fus-1, Sufu	(42)
Anti-angiogenic		
miR-92a	Integrin-α5	(43)
miR-17	JAK 1	(44)
miR-15b	VEGF	(45)
miR-16	VEGF	(45)
miR-20a	MKK3	(46)
miR-20b	HIF-1α	(47)
miR-221	c-kit	(48)
miR-222	c-kit	(48)
miR-320	IGF-1	(49)
miR-503	CCNE1, cdc25A	(50)
Remodeling		
miR-29a	TAB-1	(51)
miR-29b	Smads, β-catenin	(52)
miR-29c	Smads, β-catenin	(53)
miR-192/215	E-cadherin, SIP1	(54)

keratinocyte wound healing. Several noteworthy examples are mentioned here.

miR-21 and miR-130a

In human HaCaT keratinocytes, miR-21 was upregulated by TGF-β1, and miR-21 overexpression promoted keratinocyte migration (27). Conversely, miR-21 knockdown inhibited TGF-β1-induced keratinocyte migration, indicating that miR-21 is critical for TGF-β-driven keratinocyte migration (27). In a study conducted by Pastar et al., primary human keratinocytes were transfected with miR-21 or miR-130a expressed using pSilencer vector. They found that miR-21 and miR-130a overexpression led to suppression and downregulation of LepR and early growth response factor 3. The luciferase reporter assay verified LepR as a direct target for miR-21 and miR-130a. Both miR-21 and miR-130a delayed epithelialization in an acute human skin wound model (63).

miR-31

Li et al. identified miR-31 as a key regulator for the promotion of keratinocyte proliferation and migration in wound healing using human primary keratinocytes. The expression of miR-31 in an *in vivo* human skin wound-healing model was upregulated in keratinocytes in the inflammatory through proliferation phase at the wound edge. Overexpression of miR-31 in human primary keratinocytes encouraged proliferation and migration. Inhibition of miR-31 had opposing effects. In addition, epithelial membrane protein 1 (EMP-1) was identified as a direct target of miR-31 and silencing of EMP-1 mimicked

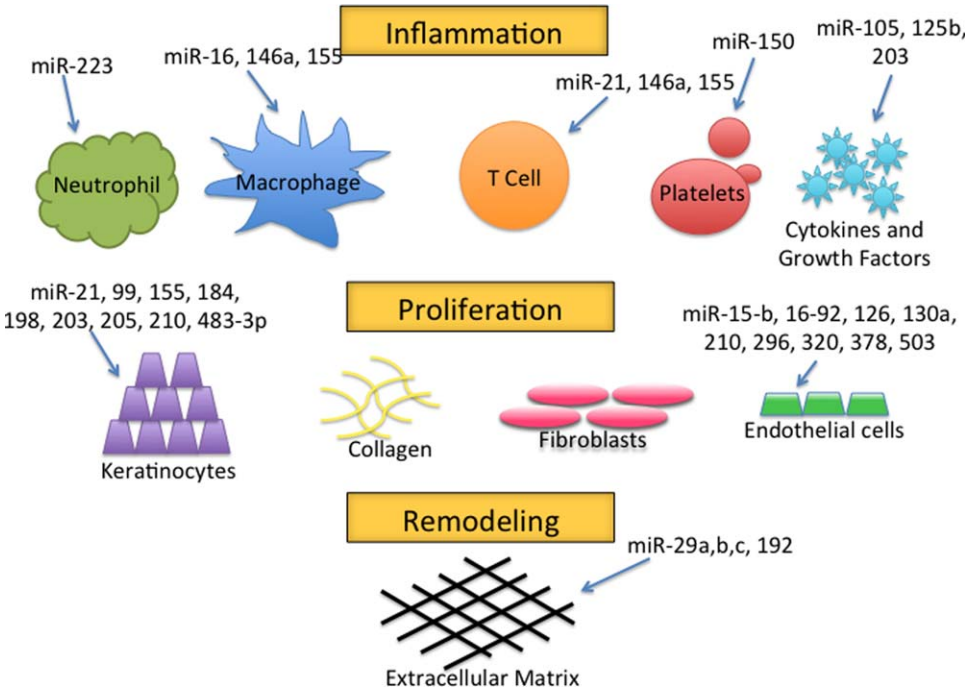

FIG 2 *Potential roles of miRNAs in wound healing. Different miRNAs are potentially involved in promoting or inhibiting wound healing during the three different phases of wound healing.*

effects of overexpression of miR-31. Lastly, TGFβ2 was found to upregulate miR-31 expression (64).

miR-99 and miR-100

Jin et al. used human immortal keratinocyte cell lines (HaCaT) and ectopically transfected miR-99a, miR-99b, miR-100, or control microRNA mimic (Dharmacon Pittsburgh, PA) with DharmaFECT Transfection Reagent 1. This led to a statistically significant downregulation in cell proliferation (measured by MTT assay) and cell migration (measured by scratch assay and trans-well assay) as compared to the cells treated with control mimic. Additionally, the levels of 63 miRNAs were changed ($P < 0.05$) (28).

miR-210

Biswas et al. tested the significance of HIF-1α in regulating E2F3 and cell proliferation in keratinocytes by human HaCaT keratinocytes. HIF-1α maintenance caused attenuated expression of E2F3, proposing that HIF-1α downregulates E2F3 *via* a miR-210-dependent pathway. Ad-VP16-HIF-1α was delivered to HaCaT keratinocytes under normoxic conditions to force stabilization of HIF-1α to examine whether HIF-1α stabilization was necessary for influencing ischemia-inducible miR210 expression. This process resulted in marked induction of miR-210 expression, demonstrating that in keratinocytes miR-210 transcription is driven by HIF-1α. Furthermore, downregulation of basal miR-210 levels in keratinocytes significantly increased cell proliferation. Therefore, it was demonstrated that in keratinocytes, HIF-1α drives miR-210 expression, which in turn compromises cell proliferation by targeting E2F3 (40).

miR-483-3p

Bertero et al. investigated miR-483-3p in scratch-injured cultures of human keratinocytes. They demonstrated upregulation of miR-483-3p in wounded normal human keratinocytes (NHKs). This expression was significantly increased at 15 h after wounding and reached a peak of induction (6–8-fold) between 24 and 72 h. Wound beds were fully closed at 24 h postinjury as visualized by light microscopy. These findings added to the hypothesis that miR-483-3p plays a role in the arrest of the wound closure process and its accumulation at the final stage peaks (34).

miRNAs Regulate *in vivo* Wound Healing Processes

Mouse models provide vital insights into the mechanisms and pathophysiology of cutaneous wound repair. There are several notable mouse models used in *in vivo* wound healing processes, including punch biopsies or excisional wounds on the necks and backs, and full-thickness wounding of mouse tails. These models compare wound healing through a variety of wound-inducing mechanisms.

miR-21

Yang et al. studied how miR-21 was upregulated during wound healing, coincident with the temporal expression pattern of TGF-β1 after 4 mm punch biopsies were made on the back of mice (27). Knockdown of endogenous miR-21 in mice through specific antagomir reduced TGF-β1 induced

keratinocyte migration and re-epithelialization in a scratch wound-healing assay. Furthermore, overexpression of miR-21 induced TGF-β1 expression and enhanced keratinocyte migration during wound healing (27). These data further suggest that miR-21 is critical for TGF-β-driven wound healing process.

miR-27b

miR-27b was studied in type 2 diabetic db/db and db/+ mice by Wang et al., and they found that miR-27b expression was decreased in db/db bone marrow-derived angiogenic cells (BMACs) (65). Furthermore, they found that miR-27b mimic rescues impaired BMAC angiogenesis through suppression of TSP-1, TSP-2, p66(shc), and semaphorin 6A and improved topical cell therapy of diabetic BMACs on diabetic skin wound closure, while normal BMAC therapy with miR-27b inhibition showed reduced efficacy in wound closure of mice with 6mm excisional wounds. Overall, these data suggest that local miR-27b delivery may improve wound healing in diabetic mice (65).

miR-146a

Xu et al. compared wound healing process in diabetic (db/db) mice with age-matched nondiabetic heterozygous ($db/^{+}$) control mice and measured the expression of miRNA-146a and its target genes before and after 8 mm punch biopsies were taken on the dorsum of the mice. miR-146a expression was found to be significantly downregulated in diabetic mouse wounds, which correlated with increased gene expression of proinflammatory target genes. The diabetic mice displayed impaired and delayed wound healing compared to nondiabetic mice. After treating mice with mesenchymal stem cells (MSCs), increased expression of miR-146a was observed leading to decreased proinflammatory target gene expression and improved wound healing. These findings demonstrated that decreased expression of miR-146a in diabetic wounds may contribute to wound healing impairment (58).

miR-155

With the creation of punch wounds in mice, Van Sollingen et al. found an increased expression of miR-155 in wounded tissue as compared to normal skin. Moreover, miR-155 knockout mice had increased wound closure compared to the wild-type mice. miR-155 knockout mice treated with interleuikin-4 showed an increase expression of finding in inflammatory zone-1 gene, which is crucial to deposition of type 1 collagen and the overall wound healing process (66).

miR-378a

Li et al. generated miR-Pirate378a (anti-miR-378a) and miR-378a transgenic mice and studied rate of wound healing. Through PCR measurement, miR-Pirate 378a expression was significantly higher and miR-378a-5p expression significantly lower in the transgenic mice. After transgenic and wild type mice were subjected to wound healing *via* cervical dermal punch biopsy, which left full-thickness excisional wounds around 5 mm on both sides of the neck, miR-Pirate378a transgenic mice displayed enhanced wound healing observed on the sixth day as compared to wild type through decreased measured wound size. This was due to the upregulation of vimentin and β3 integrin in transgenic mice, due to knockdown of miR-378a, leading to fibroblast differentiation and angiogenesis upregulation ultimately producing enhanced wound healing (67).

Future Directions and Conclusions

While the majority of the human genome is indeed noncoding, the practical implication of it is just emerging through studies on miRNAs. miRNAs are likely to regulate multiple aspects of wound healing process throughout the stages of inflammation, proliferation and remodeling of damaged skin, as summarized in Fig. 2. While many miRNAs have been identified, very few have been demonstrated *in vivo*. Understanding and identifying ways specific miRNAs regulate wound healing will uncover the potential new therapeutic targets for wounding healing, including miRNAs and its targets.

Although hurdles remain, including the actual delivery of miRNA to target tissues, due to inability to penetrate a lipid membrane, the delivery of mimic miRNAs or miRNA inhibitors to tissue has been done using four methods at least: 1) RNA oligonucleotides conjugated with other lipophilic molecules; 2) intravenous injection of antagomiRs, which are chemically modified cholester-conjugated single strand oligonucleotides; 3) the use of locked nucleic acid modified oligonucleotides (68,69); 4) through expression vectors (68). A clinical trial investigating the role of the miR-210 gene in chronic wound healing is currently underway, making science one step closer to delivering miRNA specific therapies to combat chronic, non-healing wounds. As research evolves, the ability to utilize target miRNA-based therapy can further individualize treatment for patients with non-healing wounds and reduce this healthcare burden.

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References

[1] Sen, C. K., Gordillo, G. M., Roy, S., Kirsner, R., Lambert, L., et al. (2009) Human skin wounds: a major and snowballing threat to public health and the economy. Wound Repair Regen. 17, 763–771.

[2] Brem, H., Stojadinovic, O., Diegelmann, R. F., Entero, H., Lee, B., et al. (2007) Molecular markers in patients with chronic wounds to guide surgical debridement. Mol. Med. 13, 30–39.

[3] Diegelmann, R. F. and Evans, M. C. (2004) Wound healing: an overview of acute, fibrotic and delayed healing. Front. Biosci. 9, 283–289.

[4] Stadelmann, W. K., Digenis, A. G., and Tobin, G. R. (1998) Physiology and healing dynamics of chronic cutaneous wounds. Am. J. Surg. 176, 26S–38S.

[5] Drinkwater, S. L., Smith, A., and Burnand, K. G. (2002) What can wound fluids tell us about the venous ulcer microenvironment? Int. J. Low Extrem. Wounds 1, 184–190.

[6] Miranda, K. C., Huynh, T., Tay, Y., Ang, Y. S., Tam, W. L., et al. (2006) A pattern-based method for the identification of MicroRNA binding sites and their corresponding heteroduplexes. Cell 126, 1203–1217.

[7] Ardekani, A. M. and Naeini, M. M. (2010) The role of microRNAs in human diseases. Avicenna J. Med. Biotechnol. 2, 161–179.
[8] Ha, M. and Kim, V. N. (2014) Regulation of microRNA biogenesis. Nat. Rev. Mol. Cell. Biol. 15, 509–524.
[9] Gregory, R. I., Chendrimada, T. P., and Shiekhattar, R. (2006) MicroRNA biogenesis: isolation and characterization of the microprocessor complex. Methods Mol. Biol. 342, 33–47.
[10] Lee, Y., Ahn, C., Han, J., Choi, H., Kim, J., et al. (2003) The nuclear RNase III Drosha initiates microRNA processing. Nature 425, 415–419.
[11] Murchison, E. P. and Hannon, G. J. (2004) miRNAs on the move: miRNA biogenesis and the RNAi machinery. Curr. Opin. Cell. Biol. 16, 223–229.
[12] Moreno-Moya, J. M., Vilella, F., and Simon, C. (2014) MicroRNA: key gene expression regulators. Fertil. Steril. 101, 1516–1523.
[13] Bartel, D. P. (2009) MicroRNAs: target recognition and regulatory functions. Cell 136, 215–233.
[14] Kai, Z. S. and Pasquinelli, A. E. (2010) MicroRNA assassins: factors that regulate the disappearance of miRNAs. Nat. Struct. Mol. Biol. 17, 5–10.
[15] He, L. and Hannon, G. J. (2004) MicroRNAs: small RNAs with a big role in gene regulation. Nat. Rev. Genet. 5, 522–531.
[16] Yi, R., O'Carroll, D., Pasolli, H. A., Zhang, Z., Dietrich, F. S., et al. (2006) Morphogenesis in skin is governed by discrete sets of differentially expressed microRNAs. Nat. Genet. 38, 356–362.
[17] Shilo, S., Roy, S., Khanna, S., and Sen, C. K. (2007) MicroRNA in cutaneous wound healing: a new paradigm. DNA Cell. Biol. 26, 227–237.
[18] Eberhart, J. K., He, X., Swartz, M. E., Yan, Y. L., Song, H., Boling, T.C., et al. (2008) MicroRNA Mirn140 modulates Pdgf signaling during palatogenesis. Nat. Genet. 40, 290–298.
[19] Lu, L. F., Thai, T. H., Calado, D. P., Chaudhry, A., Kubo, M., et al. (2009) Foxp3-dependent microRNA155 confers competitive fitness to regulatory T cells by targeting SOCS1 protein. Immunity 30, 80–91.
[20] Shanmugam, N., Reddy, M. A., and Natarajan, R. (2008) Distinct roles of heterogeneous nuclear ribonuclear protein K and microRNA-16 in cyclooxygenase-2 RNA stability induced by S100b, a ligand of the receptor for advanced glycation end products. J. Biol. Chem. 283, 36221–36233.
[21] Sheedy, F. J., Palsson-McDermott, E., Hennessy, E. J., Martin, C., O'Leary, J. J., et al. (2010) Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. Nat. Immunol. 11, 141–147.
[22] Benakanakere, M. R., Li, Q., Eskan, M. A., Singh, A. V., Zhao, J., Galicia, J. C., et al. (2009) Modulation of TLR2 protein expression by miR-105 in human oral keratinocytes. J. Biol. Chem. 284, 23107–23115.
[23] Xu, N., Brodin, P., Wei, T., Meisgen, F., Eidsmo, L., Nagy, N., et al. (2011) MiR-125b, a MicroRNA Downregulated in Psoriasis, Modulates Keratinocyte Proliferation by Targeting FGFR2. J. Invest. Dermatol. 131, 1521–1529.
[24] Park, H., Huang, X., Lu, C., Cairo, M. S., and Zhou, X. (2015) MicroRNA-146a and microRNA-146b regulate human dendritic cell apoptosis and cytokine production by targeting TRAF6 and IRAK1 proteins. J. Biol. Chem. 290, 2831–2841.
[25] Primo, M. N., Bak, R. O., Schibler, B., and Mikkelsen, J. G. (2012) Regulation of pro-inflammatory cytokines TNFalpha and IL24 by microRNA-203 in primary keratinocytes. Cytokine 60, 741–748.
[26] Johnnidis, J. B., Harris, M. H., Wheeler, R. T., Stehling-Sun, S., Lam, M. H., Kirak, O., et al. (2008) Regulation of progenitor cell proliferation and granulocyte function by microRNA-223. Nature 451, 1125–1129.
[27] Yang, X., Wang, J., Guo, S. L., Fan, K. J., Li, J., et al. (2011) miR-21 promotes keratinocyte migration and re-epithelialization during wound healing. Int. J. Biol. Sci. 7, 685–690.
[28] Jin, Y., Tymen, S. D., Chen, D., Fang, Z. J., Zhao, Y., et al. (2013) MicroRNA-99 family targets AKT/mTOR signaling pathway in dermal wound healing. PLoS One 8, e64434
[29] Pottier, N., Maurin, T., Chevalier, B., Puissegur, M. P., Lebrigand, K., et al. (2009) Identification of keratinocyte growth factor as a target of microRNA-155 in lung fibroblasts: implication in epithelial–mesenchymal interactions. PLoS One 4, e6718
[30] Yu, J., Peng, H., Ruan, Q., Fatima, A., Getsios, S., and Lavker, R. M. (2010) MicroRNA-205 promotes keratinocyte migration via the lipid phosphatase SHIP2. Faseb J. 24, 3950–3959.
[31] Sundaram, G. M., Common, J. E., Gopal, F. E., Srikanta, S., Lakshman, K., et al. (2013) 'See-saw' expression of microRNA-198 and FSTL1 from a single transcript in wound healing. Nature 495, 103–106.
[32] Viticchie, G., Lena, A. M., Cianfarani, F., Odorisio, T., Annicchiarico-Petruzzelli, M., et al. (2012) MicroRNA-203 contributes to skin re-epithelialization. Cell Death Dis. 3, e435
[33] Biswas, S., Roy, S., Banerjee, J., Hussain, S. R., Khanna, S., Meenakshisundaram, G., et al. (2010) Hypoxia inducible microRNA 210 attenuates keratinocyte proliferation and impairs closure in a murine model of ischemic wounds. Proc. Natl. Acad. Sci. USA 107, 6976–6981.
[34] Bertero, T., Gastaldi, C., Bourget-Ponzio, I., Imbert, V., Loubat, A., et al. (2011) miR-483-3p controls proliferation in wounded epithelial cells. Faseb J. 25, 3092–3105.
[35] Suárez, Y., Fernández-Hernando, C., Yu, J., Gerber, S. A., Harrison, K. D., et al. (2008) Dicer-dependent endothelial microRNAs are necessary for postnatal angiogenesis. Proc. Natl. Acad. Sci. USA 105, 14082–14087.
[36] Suarez, Y., Fernandez-Hernando, C., Pober, J. S., and Sessa, W. C. (2007) Dicer dependent microRNAs regulate gene expression and functions in human endothelial cells. Circ. Res. 100, 1164–1173.
[37] Wang, S., Aurora, A. B., Johnson, B. A., Qi, X., McAnally, J., et al. (2008) The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis. Dev. Cell 15, 261–271.
[38] Harris, T. A., Yamakuchi, M., Ferlito, M., Mendell, J. T., and Lowenstein, C. J. (2008) MicroRNA-126 regulates endothelial expression of vascular cell adhesion molecule 1. Proc. Natl. Acad. Sci. USA 105, 1516–1521.
[39] Chen, Y. and Gorski, D. H. (2008) Regulation of angiogenesis through a microRNA (miR-130a) that down-regulates antiangiogenic homeobox genes GAX and HOXA5. Blood 111, 1217–1226.
[40] Fasanaro, P., D'Alessandra, Y., Di Stefano, V., Melchionna, R., Romani, S., et al. (2008) MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3. The Journal of Biological Chemistry 283, 15878–15883.
[41] Wurdinger, T., Tannous, B. A., Saydam, O., Skog, J., Grau, S., et al. (2008) miR-296 regulates growth factor receptor overexpression in angiogenic endothelial cells. Cancer Cell 14, 382–393.
[42] Lee, D. Y., Deng, Z., Wang, C. H., and Yang, B. B. (2007) MicroRNA-378 promotes cell survival, tumor growth, and angiogenesis by targeting SuFu and Fus-1 expression. Proc. Natl. Acad. Sci. USA 104, 20350–20355.
[43] Bonauer, A., Carmona, G., Iwasaki, M., Mione, M., Koyanagi, M., et al. (2009) MicroRNA-92a controls angiogenesis and functional recovery of ischemic tissues in mice. Science 324, 1710–1713.
[44] Doebele, C., Bonauer, A., Fischer, A., Scholz, A., Reiss, Y., et al. (2010) Members of the microRNA-17-92 cluster exhibit a cell-intrinsic antiangiogenic function in endothelial cells. Blood 115, 4944–4950.
[45] Hua, Z., Lv, Q., Ye, W., Wong, C. K. A., Cai, G., et al. (2006) MiRNA-directed regulation of VEGF and other angiogenic factors under hypoxia. PLoS One 1, e116
[46] Pin, A. L., Houle, F., Guillonneau, M., Paquet, E. R., Simard, M. J., and Huot, J. (2012) miR-20a represses endothelial cell migration by targeting MKK3 and inhibiting p38 MAP kinase activation in response to VEGF. Angiogenesis 15, 593–608.
[47] Lei, Z., Li, B., Yang, Z., Fang, H., Zhang, G. M., et al. (2009) Regulation of HIF-1alpha and VEGF by miR-20b tunes tumor cells to adapt to the alteration of oxygen concentration. PLoS One 4, e7629
[48] Felli, N., Fontana, L., Pelosi, E., Botta, R., Bonci, D., et al. (2005) MicroRNAs 221 and 222 inhibit normal erythropoiesis and erythroleukemic cell growth via kit receptor down-modulation. Proc. Natl. Acad. Sci. USA 102, 18081–18086.
[49] Kuehbacher, A., Urbich, C., and Dimmeler, S. (2008) Targeting microRNA expression to regulate angiogenesis. Trends Pharm. Sci. 29, 12–15.
[50] Caporali, A., Meloni, M., Vollenkle, C., Bonci, D., Sala-Newby, G. B., et al. (2011) Deregulation of microRNA-503 contributes to diabetes mellitus-induced impairment of endothelial function and reparative angiogenesis after limb ischemia. Circulation 123, 282–291.

[51] Ciechomska, M., O'Reilly, S., Suwara, M., Bogunia-Kubik, K., and van Laar, J. M. (2014) MiR-29a reduces TIMP-1 production by dermal fibroblasts via targeting TGF-beta activated kinase 1 binding protein 1, implications for systemic sclerosis. PLoS One 9, e115596

[52] Li, Z., Hassan, M. Q., Jafferji, M., Aqeilan, R. I., Garzon, R., Croce, C. M., et al. (2009) Biological functions of miR-29b contribute to positive regulation of osteoblast differentiation. J. Biol. Chem. 284, 15676–15684.

[53] van Rooij, E., Sutherland, L. B., Thatcher, J. E., DiMaio, J. M., Naseem, R. H., Marshall, W. S., et al. (2008) Dysregulation of microRNAs after myocardial infarction reveals a role of miR-29 in cardiac fibrosis. Proc. Natl. Acad. Sci. USA 105, 13027–13032.

[54] Wang, B., Herman-Edelstein, M., Koh, P., Burns, W., Jandeleit-Dahm, K., et al. (2010) E-Cadherin expression is regulated by miR-192/215 by a mechanism that is independent of the profibrotic effects of transforming growth factor-β. Diabetes 59, 1794–1802.

[55] Rot, A. and von Andrian, U. H. (2004) Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. Annu. Rev. Immunol. 22, 891–928.

[56] Rodero, M. P. and Khosrotehrani, K. (2010) Skin wound healing modulation by macrophages. Int. J. Clin. Exp. Pathol. 3, 643–653.

[57] O'Connell, R. M., Rao, D. S., and Baltimore, D. (2012) microRNA regulation of inflammatory responses. Annu. Rev. Immunol. 30, 295–312.

[58] Xu, J., Wu, W., Zhang, L., Dorset-Martin, W., Morris, M. W., et al. (2012) The role of microRNA-146a in the pathogenesis of the diabetic wound-healing impairment: correction with mesenchymal stem cell treatment. Diabetes 61, 2906–2912.

[59] Midwood, K. S., Williams, L. V., and Schwarzbauer, J. E. (2004) Tissue repair and the dynamics of the extracellular matrix. Int. J. Biochem. Cell. Biol. 36, 1031–1037.

[60] Falanga, V., Zitelli, J. A., and Eaglstein, W. H. (1988) Wound healing. J. Am. Acad. Dermatol. 19, 559–563.

[61] Brancato, S. K. and Albina, J. E. (2011) Wound macrophages as key regulators of repair: origin, phenotype, and function. Am. J. Pathol. 178, 19–25.

[62] Kulshreshtha, R., Ferracin, M., Wojcik, S. E., Garzon, R., Alder, H., et al. (2007) A microRNA signature of hypoxia. Mol. Cell. Biol. 27, 1859–1867.

[63] Pastar, I., Khan, A. A., Stojadinovic, O., Lebrun, E. A., Medina, M. C., et al. (2012) Induction of specific microRNAs inhibits cutaneous wound healing. J. Biol. Chem. 287, 29324–29335.

[64] Li, D., Li, X., Wang, A., Meisgen, F., Pivarcsi, A., et al. (2015) MicroRNA-31 promotes skin wound healing by enhancing keratinocyte proliferation and migration. J. Invest. Dermatol. 135, 1676–1685.

[65] Wang, J. M., Tao, J., Chen, D. D., Cai, J. J., Irani, K., et al. (2014) MicroRNA miR-27b rescues bone marrow-derived angiogenic cell function and accelerates wound healing in type 2 diabetes mellitus. Arterioscler. Thromb. Vasc. Biol. 34, 99–109.

[66] van Solingen, C., Araldi, E., Chamorro-Jorganes, A., Fernandez-Hernando, C., and Suarez, Y. (2014) Improved repair of dermal wounds in mice lacking microRNA-155. J. Cell. Mol. Med. 18, 1104–1112.

[67] Tang, H., Jin, X., Li, Y., Jiang, H., Tang, X., et al. (2014) A large-scale screen for coding variants predisposing to psoriasis. Nat. Genet. 46, 45–50.

[68] Fasanaro, P., Greco, S., Ivan, M., Capogrossi, M. C., and Martelli, F. (2010) microRNA: emerging therapeutic targets in acute ischemic diseases. Pharm. Ther. 125, 92–104.

[69] Weiler, J., Hunziker, J., and Hall, J. (2006) Anti-miRNA oligonucleotides (AMOs): ammunition to target miRNAs implicated in human disease? Gene Ther. 13, 496–502.