The progress, prospects, and challenges of the use of non-coding RNA for diabetic wounds

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Chronic diabetic wounds affect the quality of life of patients, resulting in significant social and economic burdens on both individuals and the health care system. Although treatment methods for chronic diabetic wounds have been explored, there remains a lack of effective treatment strategies; therefore, alternative strategies must be explored. Recently, the abnormal expression of non-coding RNA in diabetic wounds has received widespread attention since it is an important factor in the development of diabetic wounds. This article reviews the regulatory role of three common non-coding RNAs (microRNA [miRNA], long non-coding RNA [lncRNA], and circular RNA [circRNA]) in diabetic wounds and discusses the diagnosis, treatment potential, and challenges of non-coding RNA in diabetic wounds. This article provides insights into new strategies for diabetic wound diagnosis and treatment at the genetic and molecular levels.

INTRODUCTION

Diabetes has become one of the main common diseases threatening public health.[1,2] Currently, there are 340 million individuals with diabetes worldwide, and this number is expected to increase to 440 million by 2030, resulting in 4.6 million deaths every year.[3,4] Approximately 25% of all patients with diabetes suffer from diabetic foot ulcers (DFUs), one of the most common associated complications, which requires repeated surgical interventions and has become the main cause of non-traumatic amputation with a 5-year mortality rate of >50%; hence, it has caused a significant burden to patients.[5–10]

Imbalances in regulatory factor activity may lead to delayed healing and consequent formation of chronic wounds,[11] such as DFUs. At present, diabetic wounds are mainly treated by controlling excessive inflammation and promoting cell proliferation. Sadly, existing treatment strategies are not satisfactory, and few alternative options exist (Figure 1).[12,13]

MicroRNAs (miRNAs) are endogenously expressed non-coding RNAs (ncRNAs) with a length of 18−22 nucleotides, and they contribute significantly to gene regulation at the post-transcriptional level.[14] Studies have shown that miRNAs are involved in diabetic wound healing by regulating the functions of repair cells, such as immune cells, endothelial cells, fibroblasts, and keratinocytes.[15] Long ncRNAs (lncRNAs) are important to many cellular processes activated in wound healing, such as cell proliferation, migration, differentiation, and apoptosis, as well as angiogenesis and re-epithelialization.[16] Additionally, circular RNA (circRNA) helps regulate the physiological and pathological processes of many diseases, including diabetic wound healing.[9,17]

At present, there are few systematic and comprehensive reports describing the function of ncRNAs in diabetic wound healing. This review summarizes what is known about the regulatory role of three ncRNAs (miRNA, lncRNA, circRNA) in diabetic wound healing, as well as its application prospects and challenges.

ncRNA

ncRNA refers to a type of RNA that does not encode protein. In the past, it has been shown to participate in a variety of biological processes and play important transcriptional and post-transcriptional regulatory functions.[18] ncRNAs are divided into two categories based on the length of the RNA chain; lncRNA have lengths greater than 200 nucleotides, and small ncRNAs (snRNA) have lengths less than 200 nucleotides. snRNAs can be subdivided into miRNAs and circRNAs.[19]

miRNAs inhibit the expression of target genes by binding to the 3′ untranslated region (3′ UTR) of the target mRNA.[20] The miRNA gene is processed and cleaved by RNA polymerase II (Pol II) enzyme and endonuclease Drosha III to produce pre-miRNA, which is
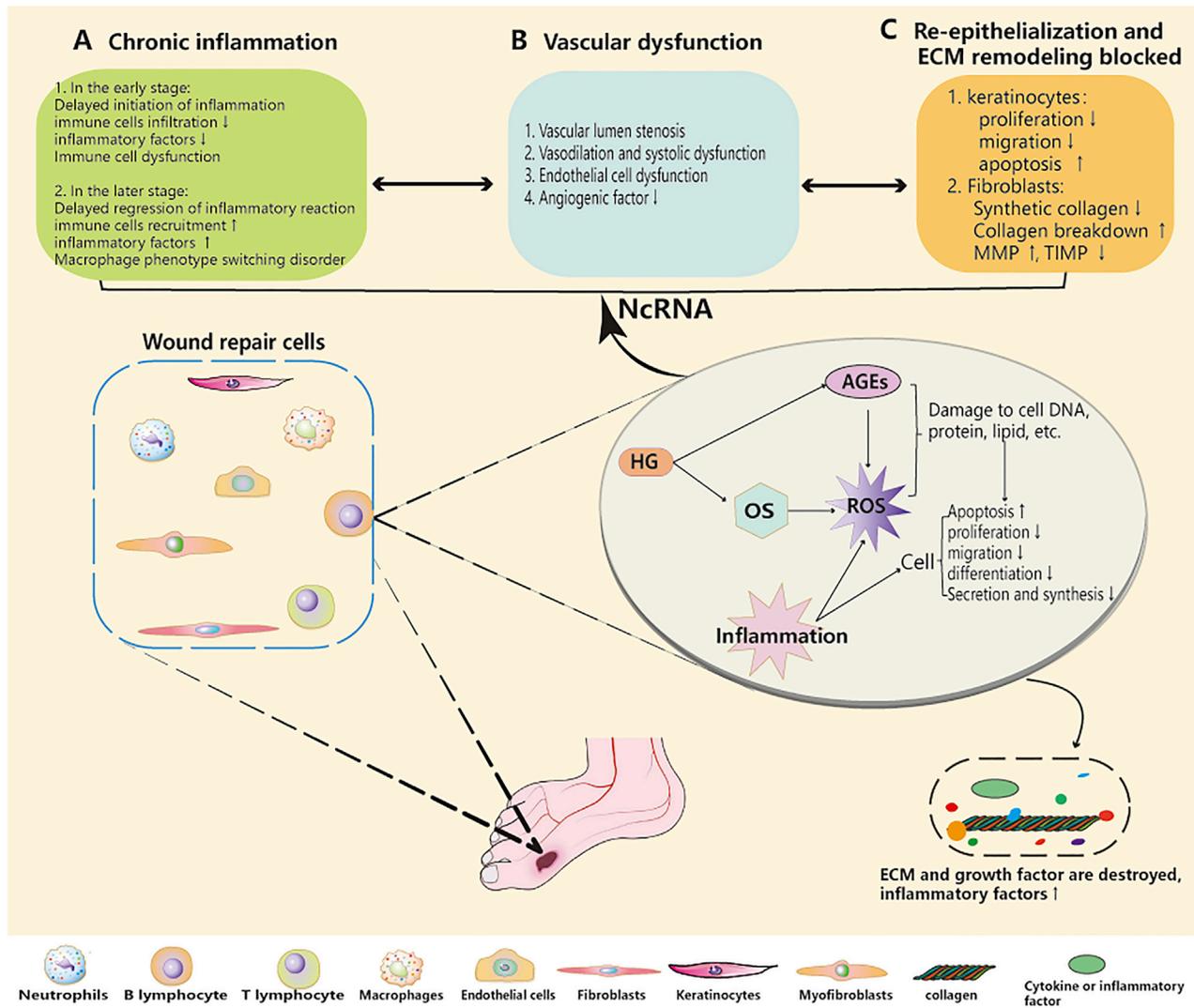
Figure 1. Pathological characteristics of diabetic wounds

(A–C) Diabetic wounds are mainly characterized by (A) chronic inflammation, (B) vascular dysfunction, and (C) delayed re-epithelization and disorder of extracellular matrix remodeling. Under the action of various factors, the function of wound-repair cells is impaired, and the extracellular matrix and its cytokines are degraded. This leads to chronic inflammation of the wound, vascular dysfunction, and obstruction of re-epithelialization and delayed extracellular matrix remodeling. All of these events also promote and reinforce each other to eventually inhibit diabetic wound repair.

transported by Exportin-5 protein to the cytoplasm, where it is digested by RNase III Dicer to produce mature miRNAs, which are loaded on Ago2 to form the large ribonucleoprotein effector complex, RNA-induced silencing complex (RISC) (Figure 2).

In the diabetic wound microenvironment, hyperglycemia, inflammatory factors, and their downstream genes can bind to miRNA promoters or affect the methylation process, thereby controlling the miRNA levels, inhibiting the activity of Dicer enzyme, and reducing the expression of related miRNAs.[20–24] Furthermore, the imbalanced miRNA can inhibit the expression of the target gene by interacting with the 3′ UTR of the target mRNA, thereby inducing mRNA degra-

dation and translation inhibition.[20,25,26] The interaction of miRNAs with other regions has also been reported, including 5′ UTR, coding sequence, methylation modification, and gene promoters.[4,25,26]

lncRNAs lack an open reading frame and can regulate gene expression at multiple levels.[27] lncRNAs play an epigenetic regulatory role by regulating methylation and histone modification;[16,28] they also interact with RNA molecules or protein complexes for transcriptional regulation[8,28,29] and can act as a molecular sponge of miRNAs to regulate downstream target genes for post-transcriptional regulation.[30–32] In addition, lncRNAs can also affect protein expression and interfere with protein binding.[33–37] Recent studies have shown
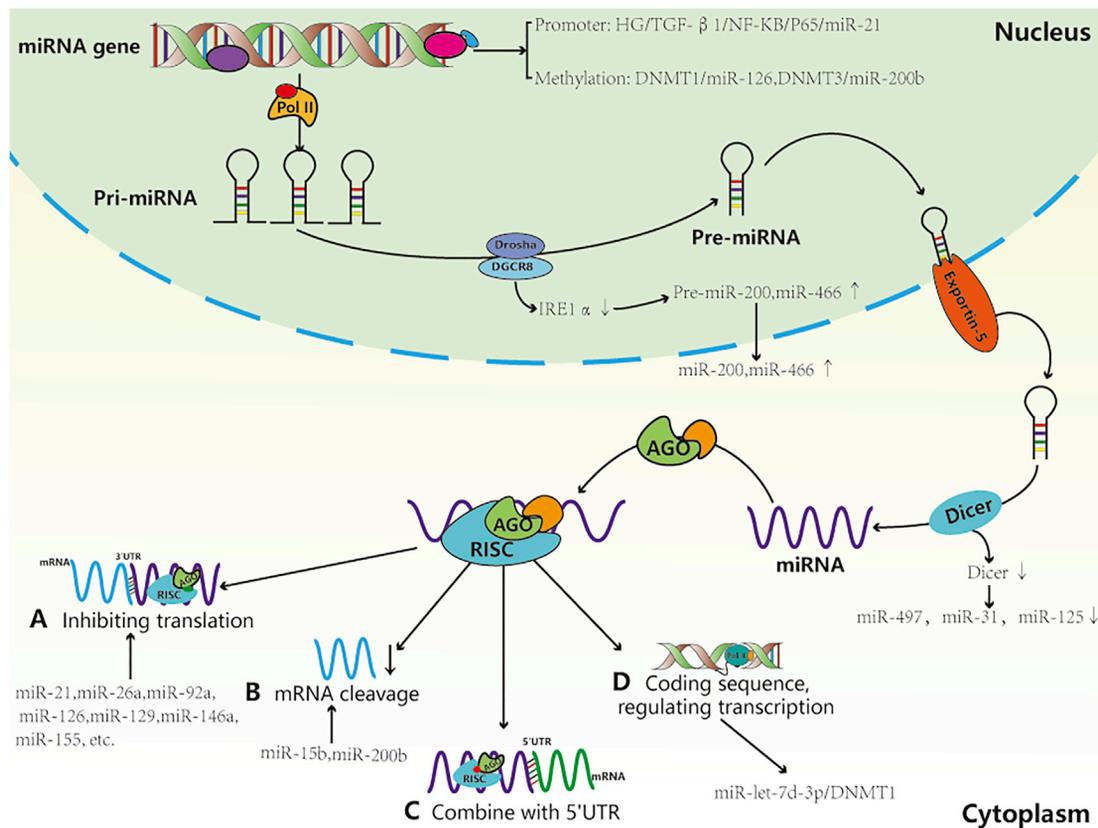
Figure 2. Biogenesis and regulation mechanism of miRNA function

(1) The miRNA gene was cleaved and processed by RNA polymerase II enzyme and endonuclease Drosha III to form pre-miRNA. The Exportin-5 protein then transports the pre-miRNA to the cytoplasm where it is digested by Dicer to produce mature miRNA, which is loaded on Ago2 to form RISC. Then, it inhibits transcription (A), regulates the degradation of mRNA (B), combine with 5′ UTR (C) and coding sequence (D) ,etc. (2) During hyperglycemia and inflammation, the effects of p65 and miR-21 promoter are enhanced, whereas miR-21 expression is induced. RNase IRE1alpha reduces the levels of miR-200 and miR-466 to that of pre-miRNA. In addition, hyperglycemia can change the expression of miRNA epigenetics by affecting the DNMT methylation process. The expression-imbalanced miRNA can inhibit the expression of the target gene by interacting with the 3′ untranslated region (3′ UTR) of the target mRNA, inducing mRNA degradation, and inhibiting translation. In addition, miRNAs can also interact with 5′ UTR and gene promoters and induce methylation modification.

that lncRNAs are key regulators of cell proliferation, differentiation, apoptosis, and other cell biological processes, thereby regulating wound healing (Figure 3).[19,27,30,38]

In circRNA, the 5′ and 3′ ends are connected to form a complete closed-loop structure. Therefore, circRNAs are resistant to degradation by the RNA exonuclease, making them more stable than linear RNAs.[39,40] circRNAs can regulate gene transcription mediated by STAT3 nuclear translocation and act as a molecular sponge of miRNA to mitigate its inhibitory effect on the downstream target genes[17,39,41]. They can also exhibit a gene regulatory function by competing with linear RNA splicing and a protein coding ability.[40,42] Furthermore, they can also interact with RNA-binding proteins,[43] RNA-processing proteins,[41,43] transcription factors, and proteases to regulate their localization and stability.[42,44,45] Recent studies have shown that circRNA dysregulation may facilitate the occurrence and development of diabetes (Figure 4).[17,43,46]

miRNAs, lncRNAs, and circRNAs can be employed as regulatory factors in the healing of diabetic wounds. miRNAs tend to act directly on mRNA of target genes and negatively regulate them, whereas lncRNAs can bind to downstream target gene mRNA and can also be used as competing endogenous RNA (ceRNA) to competitively bind miRNAs to achieve transcriptional- or post-transcriptional-level regulation. circRNA is mainly used as a molecular sponge of miRNAs to competitively inhibit miRNAs. lncRNA/circRNA/miRNA cross-link to form a huge and complex regulatory network that alters gene expression, thereby regulating multiple stages of diabetic wound repair.

The regulatory role of ncRNA in diabetic wound healing

Diabetic wounds are a type of chronic wounds associated with hyperglycemia. Their pathological changes primarily include chronic inflammation, vascular dysfunction, delayed re-epithelialization, and disorder of the extracellular matrix (ECM) remodeling. They are attributed to excessive accumulation of advanced glycation end
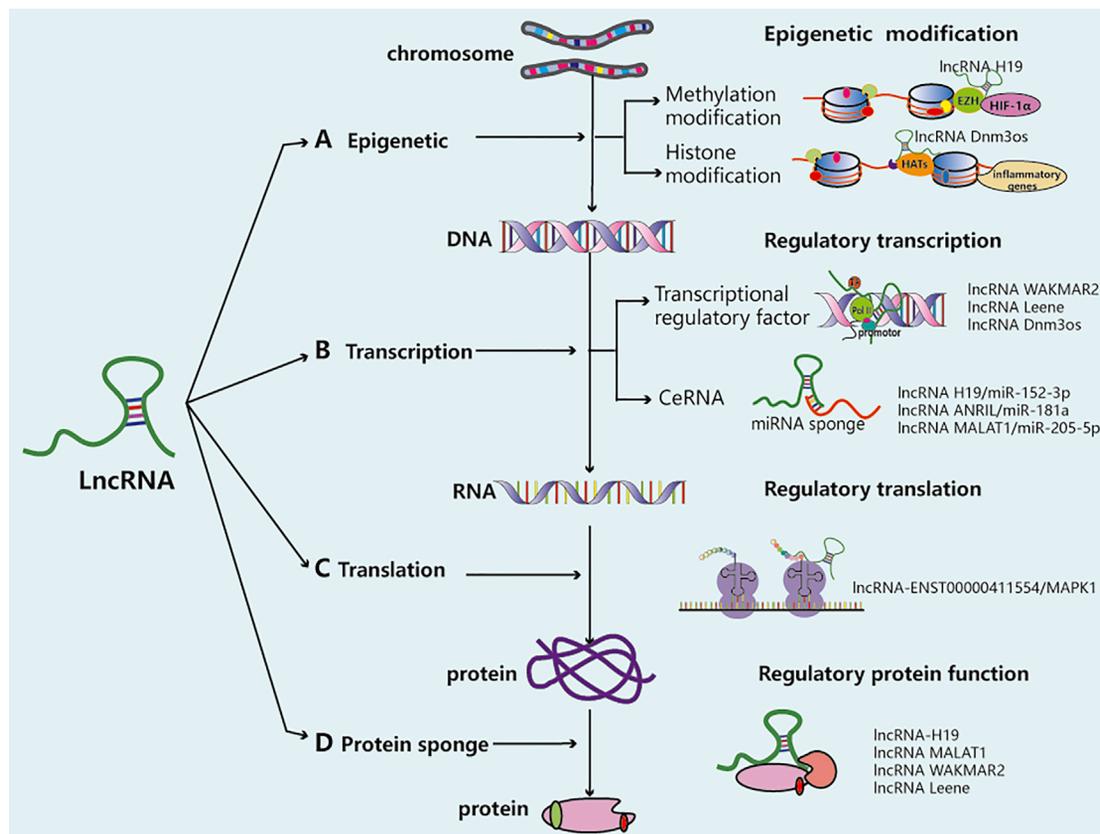
Figure 3. Overview of the mechanisms of non-coding RNA (ncRNA) function

lncRNAs can play a regulatory role in multiple levels of epigenetics (A), transcription (B), and post-transcription (C,D). HATs, histone acetyl transferases; EZH, enhancer of zeste; TF, transcription factor.

products (AGEs),[47–50] excessive oxidative stress (OS),[4,50–52] persistent inflammatory response, and imbalance of signal pathway activation.[4,47,49,51,52] Hence, they hinder immune cell function,[4,47,53] induce excessive inflammation, and damage repair cells around the wound,[48,50,54,55] all of which are accompanied with significant changes in ECM and other components,[56–59] thereby destroying the microenvironment of wound healing. Under the combined action of several complex factors, diabetic wounds can induce and exacerbate other diabetic wounds, thereby hindering the healing process of skin defects in patients with diabetes.

Previous studies have confirmed that, compared with normal wounds, the microenvironment of diabetic wounds can affect the expression level of ncRNA, which can reverse the diabetic wound microenvironment. The pathophysiological changes associated with diabetic wounds may be induced by the expression of ncRNA and its regulation imbalance of the downstream genes, which is caused by hyperglycemia, OS, inflammatory factors, and AGEs, thereby impairing cell function, resulting in chronic inflammation of the wound; angiogenesis blocked, re-epithelialization delays; and disorders of ECM remodeling.[49,50,52,53,60–62] On the contrary, when reversing its expression, NcRNA can induce the expression of related downstream

genes, which significantly reduce both the damage of the diabetic wound microenvironment to cells and the level of OS and reactive oxygen species (ROS). This ultimately will inhibit cell apoptosis, improve the level of inflammation, and promote the proliferation, migration, and differentiation of endothelial cells, keratinocytes, and fibroblasts to accelerate the process of angiogenesis, re-epithelialization, and ECM remodeling, which in turn accelerate wound healing (Figure 5).[35,49,51,52,56,63–65]

miRNAs

The microenvironment of diabetic wounds can induce an imbalance in miRNA expression by affecting the promoter, RNase activity, and methylation process.[20,22–24] Moreover, the abnormally expressed miRNA is involved in various processes of diabetic wound healing, which significantly affects the process of normal wound repair.[24,66,67]

Regulation of wound inflammation

The transition from the inflammation phase to the proliferative phase during the wound-healing process is critical.[68,69] Previous studies have shown that the imbalance of miRNA expression may explain the imbalance of wound inflammation (Table 1).

Anti-inflammatory effects

Neutrophils initiate the first stage of acute inflammation. The intrinsic defect of diabetic neutrophils may be one of the causes of chronic inflammation of DFUs, whereas microRNA (miR)-129-2-3p may be involved in neutrophil dysfunction regulation. In a previous study, it was reported that although the number of neutrophils in type 2 diabetic (T2D) mice was significantly higher than that in non-diabetic mice, the expression of miR-129-2-3p was significantly lower in the T2D mice, resulting in delayed wound healing. Contrarily, overexpression of miR-129-2-3p in the wounds of T2D mice promoted wound healing. It is possible that miR-129-2-3 directly inhibits the expression of *Casp6* and *Ccr2*, which are involved in apoptosis, inflammatory response, and chemotaxis, as well as the regulation of phagocytosis and endocytosis.[53]

Macrophage infiltration can exert an anti-inflammatory effect and remove cell debris to prevent infection in the early stages of wound healing. The late inflammatory stage transition of macrophages between pro-inflammatory and anti-inflammatory phenotypes is key to entering to the proliferative phase.[69,97] miRNAs play an anti-inflammatory regulatory role mainly by regulating the number, phenotype, and differentiation of macrophages. For example, miR-let-7b regulates macrophage polarization by inhibiting the Toll-like receptor 4 (TLR4)/nuclear factor κB (NF-κB)/STAT3/a serine/threonine kinase (AKT) signal axis.[55] Similarly, *Nox-2* increased OS, which resulted in a decrease in the expression of miR-let-7d-3p in hematopoietic stem cells of T2D mice. miR-let-7d-3p directly upregulated DNA methyltransferase (Dnmt)1, a key enzyme that mediates DNA methylation, causing *Notch1*, *PU.1*, and *Klf4* to be highly methylated, which resulted in the reduction of the number of macrophages in the wound and facilitated the polarization of macrophages to M1, thus aggravating a severe reaction. miR-let-7d-3p epigenetically inhibited the differentiation of hematopoietic stem cells into monocytes/macrophages and regulated the balance of the M1/M2 ratio in wounds, thereby affecting wound healing.[4] Interestingly, another study reported that fibroblasts in wound-granulation tissue are derived from myeloid cells such as macrophages. Keratinocyte-derived miR-21 is packaged in extracellular vesicles (EVs) and positively regulates the plasticity of wound macrophages, reverses the barriers to the conversion of myeloid cells to fibroblast-like cells in diabetic wounds, and reduces wound infiltration by macrophages, thus exerting an active anti-inflammatory effect.[76]

Keratinocytes are an important epidermal cell. Previous studies have shown that the hyperglycemia environment in diabetic skin can enhance OS and increase the inflammatory response of keratinocytes, thus stagnating the wound-healing process in the inflammatory phase.[7,71,78] Studies have confirmed that miR-132 is the key miRNA that determines the transition from inflammation to proliferation in wounds. Both *in vivo* and *in vitro* experiments have confirmed that miR-132 knockout causes severe inflammation, inhibits keratinocyte proliferation, and delays wound healing.[72] On the contrary, the topical use of miR-132 mimics on diabetic wounds can effectively improve the inflammatory response and accelerate wound closure.

Through transcriptome analysis, it was shown that miR-132 plays an anti-inflammatory role in promoting diabetic wound healing by regulating NF-κB, nucleotide-binding oligomerization domain (NOD)-like receptors, TLRs, tumor necrosis factor (TNF) signaling pathways, and other inflammation-related signaling pathways[71]. In addition, Li et al.[7] reported that miR-21 has an anti-inflammatory effect by targeting PDCD4 to regulate the NF-κB signaling pathway. In other words, miRNAs convey anti-inflammatory effects by regulating the inflammatory signals of keratinocytes and reducing the production of inflammatory factors.

Finally, inflammatory factors and chemokines also play an indispensable role in the healing of diabetic wounds. Previous studies have shown that miR-124a and miR-125b inhibit the accumulation of chemokines and cytokines in diabetic wounds, exert anti-inflammatory effects, and accelerate wound healing.[98] *In vivo* and *in vitro* studies have shown that miR-497 can reduce the levels interleukin (IL)-1β, IL-6, TNF-α, and other pro-inflammatory factors and promote wound epithelialization and granulation tissue formation, thereby accelerating the diabetic wounds-healing process.[70] Furthermore, miR-146a was significantly downregulated during the healing process of diabetic wounds, leading to a significant increase in the downstream pro-inflammatory targets *IRAK1*, *TRAF6*, and *NF-κB* and an increase in IL-6 and MIP-2 gene expression. In addition, mesenchymal stem cells (MSCs) accelerate wound healing and relieve inflammation by increasing the expression of miR-146a in diabetic wounds.[73,74] miR-146a can be coupled with cerium oxide nanoparticles to reduce wound inflammation, promote angiogenesis, and accelerate wound healing.[74] In conclusion, miRNAs can directly reduce the production of inflammatory factors and chemokines to exert anti-inflammatory effects.

Pro-inflammatory effects

Overexpression of miRNAs may be one reason for the chronic inflammation of diabetic wounds. miR-155, a pro-inflammatory miRNA mainly expressed in immune cells, is significantly upregulated in diabetic skin, and the use of miR-155 inhibitors on wounds can reduce wound inflammation. miR-155 inhibition reduced the infiltration of inflammatory cells such as neutrophils, macrophages, and T cells on the wound, whereas increasing the number of M2 macrophages.[47] The inhibitory effect on its target gene (*FGF-7*) was reversed, stimulating keratinocyte migration and proliferation, increasing vascular remodeling, and accelerating wound closure.[57] In addition, hyperglycemia can induce the expression of *NOX2* and the production of ROS by activating the miR-21/phosphatidylinositol 3-kinase (PI3K)/NOX2/ROS signaling pathway, thus increasing the inflammatory response. These results suggest that an imbalance in miR-21 expression may be one of the reasons for the continuous polarization and abnormal inflammation of M1 macrophages in diabetic wounds.[77]

Previous studies have shown that miR-15b-5p can deter DNA repair and pro-inflammatory effects by inhibiting downstream target genes, such as DNA repair genes *WEE1*, *RAD50*, *MSH2*, and *KIT*, and
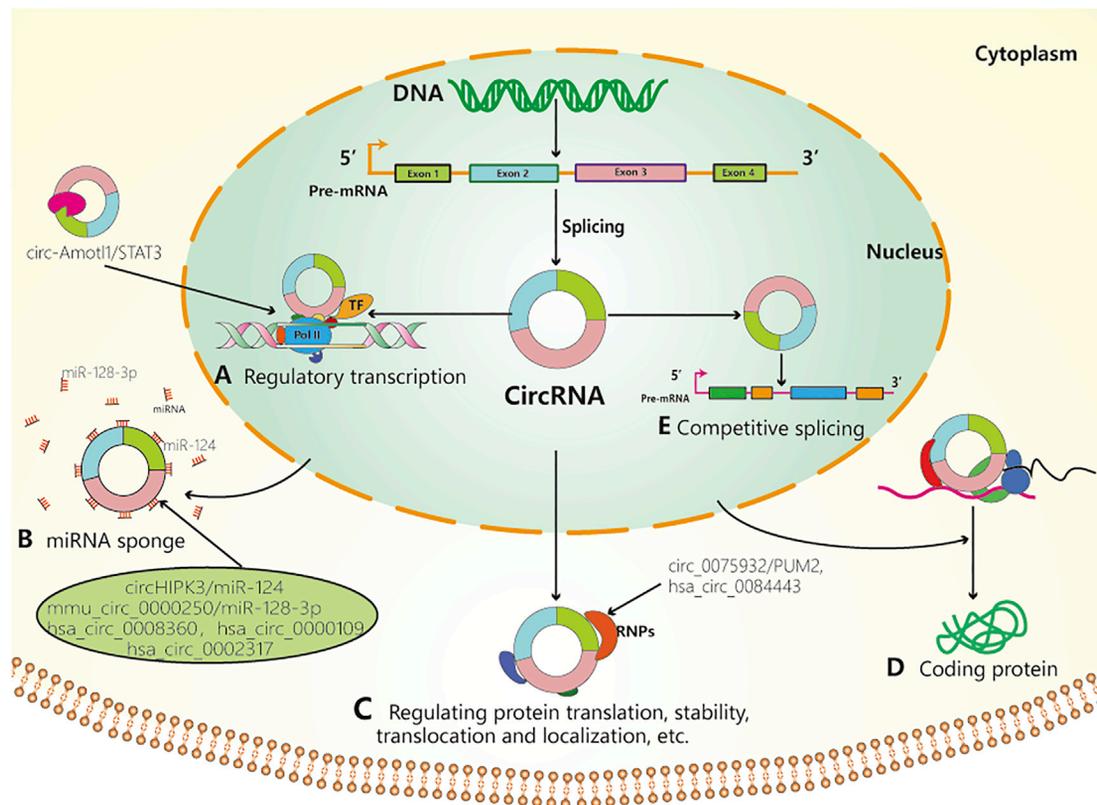
Figure 4. Mechanisms of circRNA function

circRNAs can regulate gene transcription mediated by STAT3 nuclear translocation (A) and act as competitive endogenous RNAs to mitigate the inhibitory effect of miR-124, miR-128-3p, and other miRs on the downstream target genes to regulate angiogenesis (B). circRNAs can also regulate genes by competing with linear RNA splicing (E), while having protein-coding roles (D). In addition, they can also bind to certain proteins, such as circ_0075932 and hsa_circ_0084443, and interact with others to play a regulatory role (C).

trauma inflammatory response target genes *IKBKB* and *FGF2*.[75] Therefore, imbalanced miRNAs play a pro-inflammatory role in wound repair by regulating the function of immune cells and activating inflammatory response signals.

Angiogenesis regulation

Angiogenesis is another key process in wound repair, which is necessary for the delivery and supply of nutrients to the wound so as to promote the healing process.[83,99] miRNA can participate in the process of angiogenesis by regulating the function of endothelial cells and the expression of angiogenesis-related factors. miRNA may promote anti-angiogenesis or angiogenesis in wound healing.

Angiogenesis promotion

miRNAs promote vascular endothelial cell proliferation, migration, and angiogenesis by regulating angiogenic growth factors. In contrast, the downregulation of the expression of some miRNAs in diabetic wounds may inhibit angiogenesis. TSP-1 has been shown to inhibit the adhesion and proliferation of several types of endothelial cells as well as tubular formation.[66,100] In damaged bone marrow-derived angiogenic cells (BMACs) in a diabetic mouse model (db/db), miR-

27b expression was decreased, whereas TSP-1 expression was increased. miR-27b improved the proliferation, adhesion, and angiogenesis of db/db BMACs, reduced OS, and protected BMAC function. Additionally, miR-27b directly targeted TSP-1, TSP-2, p66shc, and semaphorin6 to promote angiogenesis and increase blood perfusion and accelerate the closure of diabetic skin wounds.[51] Similarly, miR-18a/19a can accelerate the angiogenesis of diabetic wounds by downregulating TSP-1 and promoting endothelial cell migration and tubular formation.[66] Similarly, miR-23a inhibited the expression of IRF-1 and reduced the expression of inducible nitric oxide synthase (iNOS) and the angiogenesis-related factor vascular endothelial growth factor (VEGF), thus inhibiting angiogenesis and the healing process of diabetic wounds.[78] Another study showed that miR-221-3p increased the expression of VEGF and promoted cell proliferation and angiogenesis. Bioinformatics analysis indicated that miR-221-3p may be involved in AGE-receptor for AGE (RAGE) signaling, cell cycle, and p53 signaling in diabetes.[48]

miR-126 is an important molecule that regulates angiogenesis. It has been shown that the expression of miR-126 is significantly reduced in the peripheral blood of DFU patients. The abnormal expression of

miR-126 is closely related to the pathological changes in DFUs.[101] Overexpression of miR-126 can reduce the production of ROS and apoptosis in the blood-brain barrier of patients with diabetes, activate the migration and recruitment of endothelial cells to the injured site, and accelerate tissue repair.[102] Under high glucose (HG) levels, miR-126-3p in human umbilical vein endothelial cells (HUVECs) was downregulated, which increased the expression of DNMT1, an anti-angiogenic DNMT, leading to HUVEC migration dysfunction.[21] Previous studies have shown that exosomes (exos) derived from synovium MSCs overexpressing miR-126-3p can significantly activate PI3K/AKT and mitogen-activated protein kinase (MAPK)/extracellular signal-regulated kinase (ERK) pathways, which can activate endothelial cell proliferation, migration, and angiogenesis *in vitro* and stimulate angiogenesis and re-epithelialization *in vivo* to accelerate wound healing.[79]

Finally, miRNAs can also promote angiogenesis by regulating stem cell functions. Previous studies have shown that human adipose-derived stem cells (hADSCs) are damaged in patients with diabetes, which is manifested by a decrease in the expression of *miR-1248* and an increase in the expression of *CITED2*, an inhibitor of hypoxia-inducible factor 1α (HIF-1α). *CITED2* also regulates stem cell activity and differentiation and inhibits angiogenesis, thus affecting the secretion of growth factors and cell proliferation and delaying wound healing.[80] Therefore, miRNAs mainly modulate the function of endothelial cells, change the expression of angiogenic factors, and act on regulatory signals to promote angiogenesis.

Anti-angiogenesis

Abnormal expression of miRNAs may be conducive to the inhibition of angiogenesis in diabetic wounds. The expression of some anti-angiogenic miRNAs is significantly increased in diabetic wounds; contrarily, the inhibition of these miRNAs may significantly accelerate wound healing. Studies have found that inhibiting the expression of anti-angiogenic miR-92a may improve blood perfusion in ischemic diseases. MRG-110, an miR-92a inhibitor, can be administered intracutaneously in db/db mouse wounds to accelerate the formation and re-epithelialization of granulation tissue without causing systemic toxic damage.[81] A similar effect was observed in the wound model of piglets.[81] Lucas et al.[82] developed a light-induced anti-miR-92a therapeutic approach to promote healing in diabetic mice using light-resistant protective groups. The result of their study showed that the target of light-induced anti-miR-92a was more accurate and inhibited the expression of miR-92a in skin but did not significantly affect the expression of miR-92a in other organs. Light activation of anti-miR-92a stimulated wound cell proliferation and angiogenesis and significantly accelerated the healing of diabetic mice, and its effect was similar to that of traditional anti-miRNAs.[82]

In addition, miR-26a can inhibit the proliferation and migration of endothelial cells and inhibit angiogenesis by downregulating the target gene *SMAD1* and upregulating the cell cycle inhibitor p27. In contrast, local administration of the miR-26a inhibitor locked nucleic acid (LNA)-anti-miR-26a to wounds can induce angiogenesis and accelerate

wound healing, but these effects are unrelated to changes in the ratio of M1/M2 macrophages.[85] Similarly, the inhibition of miR-152-3p can upregulate phosphatase and tensin (PTEN) and promote the healing of diabetic wounds.[93] Interestingly, the expression of circulating exos or miR-20b-5p in T2D patients was significantly upregulated, indicating that miR-20b-5p or patients with diabetes exos can hinder angiogenesis and delay wound healing; contrarily, knocking out miR-20b-5p accelerated wound healing. miR-20b-5p may inhibit the angiogenesis of HUVECs by inhibiting Wnt9b/β-catenin signaling.[103]

miRNAs can exert an anti-vascular effect by regulating the expression of angiogenic factors. SDF-1α is commonly targeted by miRNAs of the miR-23 family. Studies have shown that SDF-1α was significantly decreased in the blood and tissue sections of DFU patients, and the expression of miR-23a and miR-23b was downregulated, whereas the expression of miR-23c was increased. Thus, elevated miR-23c negatively regulated SDF-1α and inhibited angiogenesis. miR-23c is also involved in the downregulation of the expression of other angiogenic factors, such as endothelial NOS (eNOS), HIF-1α, and VEGF, thus inhibiting angiogenesis.[84]

miR-205-5p inhibits VEGF protein translation by interacting with the 3′ UTR of VEGF mRNA. In contrast, anti-miR-205-5p significantly increased the expression of VEGF in MSCs, thus promoting angiogenesis.[86] Previous studies have shown that the expression of angiogenic factors HIF-1α and VEGF can be downregulated by miR-217, thus hindering the healing of foot ulcers in mice;[88] miR-217 is upregulated in the serum of DFU patients and positively correlated with Wagner grade. Further research showed that HIF-1α is the direct target gene of miR-217. Inhibition of miR-217 can activate the HIF-1α/VEGF pathway, reduce inflammation, and promote angiogenesis.[88] miR-15b is considered a key negative regulator of angiogenesis and mainly inhibits the expression of HIF-1α.[91,92] In addition, it was reported that miR-615-5p may inhibit the VEGF/AKT/eNOS signaling pathway and inhibit angiogenesis by targeting insulin growth factor 2 (IGF2) and RASSF2 in endothelial cells. Meanwhile, animal experiments found that the miR-615-5 inhibitor significantly increased angiogenesis, granulation tissue thickness, and wound-closure rate in db/db mice.[89] These miRNAs inhibit angiogenesis in tissue repair by inhibiting VEGF-related signaling pathways.

Previous studies have found that miR-200b was downregulated immediately and temporarily in endothelial cells around the wound, thus eliminating the negative regulation of miR-200b on GATA2 and VEGFR2 and initiating the process of angiogenesis; however, in diabetic wounds, the TNF-α-mediated miR-200b-GATA2-VEGFR2 signal axis exerted an anti-angiogenic effect and delayed wound healing, which was reversed after neutralizing TNF-α.[61] miR-200b can also initiate diabetic vascular disease through epigenetic modification; that is, HG reduces DNMT1 and DNMT3A, key enzymes for epigenetic modification, leading to endothelial dysfunction, which encompasses decreased endothelial nitric oxide, decreased low-density lipoprotein (LDL) uptake, and Matrigel tube-formation disorder.[22] Moreover, miR200b can inhibit angiogenesis and delay the healing
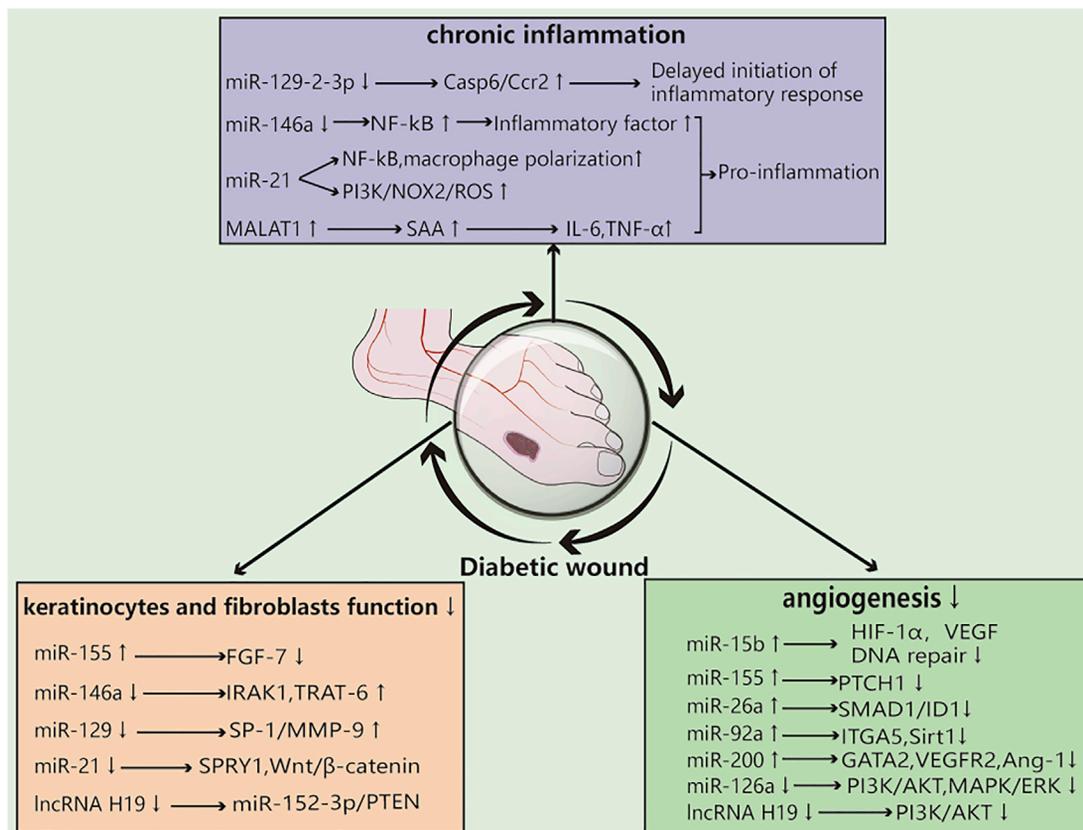
Figure 5. Regulatory effects of certain common ncRNAs on the pathology of diabetic wounds

The diabetic wound microenvironment induces ncRNA changes, which in turn, causes an imbalance in the downstream gene expression, thereby impairing the functions of immune cells, endothelial cells, keratinocytes, and fibroblasts in the wound and causing cytokine imbalance, further exacerbating the diabetic wound. Moreover, these three pathological processes are inter-related.

of diabetic wounds by inhibiting the angiogenic factors Ang-1 and ANGPT1.[66,92] These studies suggest that miRNA prevented angiogenesis in diabetic wounds by targeting ANGPT1.

HG level induced decreased endothelial cell activity, migration, and angiogenesis and increased levels of lactate hydrogenase, ROS, and cell apoptosis.[54] miRNAs can inhibit the proliferation and migration of endothelial cells, promote apoptosis, and hinder angiogenesis in diabetic wounds. Pro-inflammatory stress increases the secretion of miR-191 or miR-200b in endothelial cells, and miR-191 targets the downregulation of zonulaoccludans-1 (zo-1), which ultimately affects migration of dermal endothelial cells or fibroblasts in patients with diabetes.[90] miR-135a-3p, an anti-angiogenic miRNA, inhibited the proliferation, migration, and tube-like formation of endothelial cells in the matrix gel. miR-135a-3p effectively regulated the p38 signal stimulated by VEGF by targeting HIP1, thus playing an anti-angiogenic role.[87] Similarly, miR-155 directly targeted the 3′ UTR region of PTCH1, inhibited the expression of PTCH1, and mediated endothelial cell dysfunction.[54] In other words, these miRNAs impair the function of endothelial cells by inhibiting their proliferation and migration to achieve anti-angiogenesis.

Regulatory re-epithelialization

Keratinocytes are important cells for re-epithelialization, and dysregulated miRNAs mainly regulate wound epithelialization by affecting their functions. Studies have found that hADSC-exos overexpressing miR-21-5p promoted the migration and proliferation of keratinocytes *in vitro*. Studies on the wound model of diabetic rats showed that hADSC-exos significantly accelerated wound healing after 15 days of intervention. Histological examination revealed that hADSC-exos not only significantly promoted re-epithelialization but also angiogenesis and collagen remodeling. miR-21-5p promoted the proliferation and migration of keratinocytes and accelerated the healing of diabetic wounds by activating the Wnt/β-catenin-7 signaling pathway.[56] In addition, Li et al.[49] showed that miR-5591-5p reduced ROS production and cell apoptosis in ADSCs by targeting the AGE/AGER/JNK signal axis, increased the survival rate of ADSCs, and promoted wound re-epithelialization, accelerating wound healing in diabetics. These results suggest that miRNA is delivered through exos to activate the function of keratinocytes and promote re-epithelialization of skin wounds.

Matrix metallopeptidases (MMPs) play an indispensable role in the movement of keratinocytes. They regulate the imbalance between the

Table 1. Functions and mechanisms of miRNAs in diabetic wounds

Regulatory effect	miRNA	Expression change	Regulation mechanism	Experimental model	Mechanism of non-coding RNA (ncRNA) change	Reference
Pro-inflammatory	miR-497	↓	downregulates pro-inflammatory factors such as IL-1, IL-6, and tumor necrosis factor (TNF)-α and exerts an anti-inflammatory effect	type 1 diabetic mice	liposome transfection	70
	miR-129-2-3p	↓	directly targets and negatively regulates *Casp6* and *Ccr2* and participates in the regulation of inflammation, apoptosis, chemotaxis, phagocytosis, and endocytosis in neutrophils	type 2 diabetic mice (db/db)	plasmid transfection	53
	miR-132	↓	regulates inflammation-related signaling pathways such as the NF-κB, Toll-like receptor (TLR), NOD-like receptor, and TNF signaling pathways	human *ex vivo* skin wounds + type 2 diabetic mice (db/db)	direct application of miR-132-3p mimic	71
	miR-132	↑	inhibits the NF-κB pathway in keratinocytes to reduce the production of chemokines and the ability to attract leukocytes; also promotes the proliferation of keratinocytes by increasing the activity of STAT3 and ERK pathways	human *ex vivo* skin wounds	direct application of anti-miR-132	72
	miR-146a	↓	inhibits the NF-κB signaling pathway and reduces the expression levels of downstream inflammatory genes to exert an anti-inflammatory effect	type 2 diabetic mice (db/db)	MSC transplantation	73,74
	miR-15b-5p	–	inhibits the downstream target genes *IKBKB*, *WEE1*, *FGF2*, *RAD50*, *MSH2*, and *KIT* and participates in the regulation of DNA repair and inflammatory response processes	human *ex vivo* skin wounds	–	75
	miR-21	–	activates the miR-21/PDCD4/NF-κB signaling pathway and plays an antibacterial, anti-inflammatory, and proliferative role	*in vitro*	–	7
	miR-21	↓	EVs in keratinocytes are rich in miR-21, which promotes the conversion of macrophages into fibroblast-like cells to promote wound healing.	LysM^{Cre}-Rosa$^{mT/mG}$ mice	lipid nanoparticles	76
	let-7b	–	promotes M$_2$ polarization of macrophages and improves wound inflammation by activating the TLR4/NF-κB/STAT3/AKT signal axis	type 1 diabetic rats	plasmid transfection	55
	miR-let-7d-3p	↓	can reduce the hypermethylation of Notch1, PU.1, and Klf4 by inhibiting Dnmt1, thereby inhibiting the polarization of M1 macrophages and improving inflammation	type 2 diabetic mice (db/db)	lentiviral transfection	4

Table 1. Continued

Regulatory effect	miRNA	Expression change	Regulation mechanism	Experimental model	Mechanism of non-coding RNA (ncRNA) change	Reference
Anti-inflammatory	miR-155	–	Pro-inflammatory effect and its inhibition can reduce the infiltration of neutrophils and macrophages, reduce wound inflammation, and promote angiogenesis.	type 1 diabetic rats	direct application of miR-155 inhibitor	47,57
	miR-21	↑	The high glucose (HG)/miR-21/PI3K/NOX2/reactive oxygen species (ROS) signaling pathway induces the expression of NOX2 and the production of ROS, leading to continuous polarization of M1 macrophages and abnormal inflammation of diabetic wounds.	type 1 diabetic rats	transfection reagents	77
Promotes angiogenesis	miR-23a	↓	miR-23a and activating the IRF-1/iNOS signal axis to promote angiogenesis	type 1 diabetic rats	plasmid transfection	78
	miR-27b	↓	miR-27b can improve oxidative stress, activate internal cell function, and promote angiogenesis by inhibiting the expression of TSP-1, Sema6A, and p66shc.	type 2 diabetic mice (db/db)	transfection reagents	51
	miR-126-3p	↓	H2S induces DNMT1 expression and stimulates the expression of miR-126-3p, which promotes the migration of HUVECs.	type 2 diabetic mice (db/db)	–	21
	miR-126-3p	–	significantly activates the PI3K/AKT and MAPK/ERK pathways, accelerates re-epithelialization, activates angiogenesis, and promotes collagen maturation	type 1 diabetic rats	lentiviral transfection	79
	miR-221-3p	–	participates in regulation of the AGE-RAGE signaling pathway, p53 signaling pathway, and cell cycle-related pathways; increases the expression of angiogenic factors; and promotes angiogenesis	type 1 diabetic mice	direct application of miR-221-3p	48
	miR18a/19a	–	targets TSP-1 and promotes angiogenesis in diabetic wounds	*in vitro*	lipofectamine	66
	miR-1248	↓	miR-1248/CITED2/HIF-1α pathway dysregulation impairs the function of hADSCs, suppresses its role in promoting angiogenesis, and slows down wound repair.	type 1 diabetic rats	direct application of miR-1248 mimic or inhibitor	80

Table 1. Continued

Regulatory effect	miRNA	Expression change	Regulation mechanism	Experimental model	Mechanism of non-coding RNA (ncRNA) change	Reference
	miR-92a-3p	↑	miR-92a-3p targets inhibition of the pro-angiogenic factor ITGA5, inhibits endothelial cell (EC) migration, and hinders angiogenesis and wound repair.	type 2 diabetic mice (db/db)	synthesis miR-92a-3p inhibitor: MRG-110	81
	miR-92a	↑	Anti-miR-92a downregulates the expression of ITGA5 and Sirt1 and promotes the proliferation and angiogenesis of wound cells.	type 2 diabetic mice (db/db)	light-inducible anti-miR-92a	82
	miR210	–	Anti-miR210 acts on the downstream target genes *E2F3* and *HSP-1* to increase the proliferation and migration of keratinocytes *in vitro*, increases angiogenesis, and accelerates wound healing *in vivo*.	type 2 diabetic mice (db/db)	direct application of anti-miR210	83
	miR-23c	↑	Elevated miR-23c levels delay DFU healing by inhibiting downstream SDF-1α/CXCL12 signaling and inhibiting angiogenesis.	examined DFU tissue specimen + *in vitro*	–	84
	miR-26a	↑	inhibits the proliferation and migration of ECs and inhibits angiogenesis by regulating the BMP/SMAD1/ID1 signaling pathway	type 2 diabetic mice (db/db)	lipofectamine transfection or direct application of LNA-anti-miR-26a	85
Anti-angiogenesis	miR-20b-5p	↑	negatively regulates Wnt9b/β-catenin signaling and inhibits EC function, hinders angiogenesis, and delays wound healing	diabetic exosomes (exos) + type 1 diabetic mice	gene recombination	86
	miR-135a-3p	↑	By targeting and regulating the VEGF-HIP1-p38 signaling pathway, miR-135a-3p inhibits the proliferation, migration, and angiogenesis of ECs.	*in vitro*	lipofectamine transfection	87
	miR-155	↑	directly targets and negatively regulates PTCH1; reduces the level of nitric oxide; and increases lactate hydrogenase, cell apoptosis, and ROS, thereby inhibiting the viability, migration, and tube formation of ECs	*in vitro*	–	54
	miR-200b	↑	TNF inhibits the expression of GATA2-VEGFR2 by inducing the expression of miR-200b to hinder angiogenesis.	*in vitro*	–	61
	miR-200b	↑	HG induces reduction of the methylation of the miR-200b promoter, which leads to EC dysfunction and inhibits angiogenesis.	type 2 diabetic mice (db/db)	plasmid transfection	22
	miR-205-5	–	inhibits VEGF expression and angiogenesis and delays wound healing by binding to the 3′ UTR of *VEGF* mRNA	type 1 diabetic mice	plasmid transfection	86

Table 1. Continued

Regulatory effect	miRNA	Expression change	Regulation mechanism	Experimental model	Mechanism of non-coding RNA (ncRNA) change	Reference
	miR-217	↑	regulates the HIF-1/VEGF pathway, increases the expression levels of angiogenic factors, and reduces the expression levels of inflammatory factors	examined human DFU serum specimen + type 1 diabetic rats	direct application miR-217 inhibitors	88
	miR-615-5p	↑	targets IGF2-2 and RASSF2-2, inhibits the VEGF-AKT/eNOS signaling pathway of vascular ECs, and hinders angiogenesis	type 2 diabetic mice (db/db)	direct application of LNA-anti-miR-615-5p	89
	miR-191, miR-200b	↑	miR-191 targets and downregulates the target area zonulaoccludans-1 (ZO-1); affects the proliferation, migration, and apoptosis of diabetic dermal ECs or fibroblasts; hinders angiogenesis; and delays wound repair.	examined human diabetic tissue specimen + in vitro	—	90
	miR-466, miR200	↑	miR-466 and miR-200 directly target the angiogenic factor ANGPT1 to inhibit its expression, leading to the obstruction of angiogenesis.	type 2 diabetic mice (db/db)	—	23
	miR-15b	↑	blocks angiogenesis by inhibiting the expression of downstream pro-angiogenic factors, including VEGF and HIF-α	type 2 diabetic mice (db/db)	MSC transplantation	91
	miR-15b, miR-200	↑	inhibits the expression of downstream factors VEGF and angiopoietin-1, respectively, and inhibits angiogenesis	type 2 diabetic mice (db/db)	direct application of anti-miR15b and anti-miR200b	92
	miR-152-3p	↑	miR-152-3p antagonists can remove the targeted inhibition of PTEN, restore the function of HUVECs, and promote wound repair.	type 2 diabetic mice (db/db)	direct application of anti-miR-152-3p	93
Promotes re-epithelialization	miR-21	↓	The activation of the Wnt/β-catenin/MMP-7 pathway can promote the proliferation and migration of keratinocytes and promotes diabetic wound healing by promoting re-epithelialization, collagen remodeling, and angiogenesis in vivo.	type 1 diabetic rats	electroporation introduces miR-21 into ADSC-EXO	56
	miR-129, miR-335	↓	downregulates the expression of MMP-9 by inhibiting the transcription factor Sp1, which promotes the migration of keratinocytes and accelerates re-epithelialization	examined DFU tissue specimen + type 1 diabetic rats	liposome transfection	60

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Table 1. Continued

Regulatory effect	miRNA	Expression change	Regulation mechanism	Experimental model	Mechanism of non-coding RNA (ncRNA) change	Reference
	miR-5591-5p	↓	inhibits the AGES/AGER/JNK signaling pathway in a targeted manner, improves the survival rate of ADSCs, enhances the therapeutic effect of ADSCs, accelerates re-epithelialization, and promotes wound repair	type 1 diabetic mice	plasmid transfection	49
Anti-re-epithelialization	miR-155	↑	plays a key role in the proliferation and migration of keratinocytes and wound repair by targeting the negative regulation of FGF7, which destroys the re-epithelialization of diabetic wounds	type 1 diabetic mice	direct application of miR-155 inhibitors	57
	miR-203	↑	inhibits the epithelial-mesenchymal transition (EMT) process by inhibiting regulation of the IL-8/AKT/Slug pathway to hinder wound healing	type 1 diabetic rats	–	94
	miR-203	↑	regulates the proliferation and differentiation of keratinocytes	examined DFU tissue specimen	–	14
	miR210	–	Anti-miR210 acts on downstream target genes *E2F3* and *HSP-1* to increase the proliferation and migration of keratinocytes, thereby promoting re-epithelialization and accelerating wound healing.	type 2 diabetic mice (db/db)	direct application of anti-miR210	83
Promotes extracellular matrix (ECM) remodeling	miR-21	–	The TGF-β1-NF-κB-miR-21 signal axis induces fibroblasts to express miR-21 to regulate fibroblast function.	*in vitro*	–	20
	miR-21	–	miR-21 regulates the migration, differentiation, and contraction of fibroblasts and induces the pro-angiogenesis process of ECs. It can also promote the transformation of fibroblasts into myofibroblasts and mediates pro-inflammatory responses.	*in vitro*	plasmid transfection	95
	miR-21	↓	By activating the Wnt/β-catenin/MMP-7 pathway, miR-21 promotes the proliferation and migration of keratinocytes *in vitro* and promotes re-epithelialization, collagen remodeling, and angiogenesis in mice to promote diabetic wound healing.	type 1 diabetic rats	liposome transfection	56
	miR-21-3p	↓	directly targets SPRY1 to enhance the function of fibroblasts and increase collagen synthesis, thereby regulating ECM remodeling to speed up the healing of diabetic wounds	C57BL/6 mice	lipofectamine transfection or direct application of agomiR-21-3p	64

(Continued on next page)

Table 1. *Continued*

Regulatory effect	miRNA	Expression change	Regulation mechanism	Experimental model	Mechanism of non-coding RNA (ncRNA) change	Reference
	miR-29b	↓	MSCs upregulate miR-29b to target the expression of MMP-9 and increase the expression level of type I collagen to promote wound healing.	*in vitro*	MSC transplantation	62
Anti-ECM remodeling	miR-378a	−	Antisense miR-378a promotes angiogenesis by upregulating integrin β-3 and upregulates vimentin to promote the migration and proliferation of fibroblasts to accelerate wound healing.	transgenic anti-miR-378 mouse	gene recombination	96
	miR-29a	↑	MSCs increase the synthesis of collagen by reducing the level of miR-29a, thereby correcting the damaged biomechanical properties of diabetic skin.	type 2 diabetic mice (db/db)	direct application miR-29a	59
	miR-106b-5p	↑	reduces the expression of ERK1/2 to trigger fibroblast autophagy, reduce collagen synthesis, and increase collagen degradation, thereby delaying wound healing	rats	AGEs-sEVs	58
	miR-27-3p	↑	Elevated miR-27-3p inhibits fibroblast function and reduces collagen deposition by targeting *NOVA1*, resulting in delayed wound healing.	examined diabetic skin samples + C57BL/6 mice	plasmid transfection	65

↓, decreased expression in diabetes; ↑, increased expression in diabetes; −, not mentioned in the article.

synthesis and degradation of ECM and influence the re-epithelialization and remodeling of wounds. Studies have shown that DFU healing gradually improved with a slow decline in MMP-9 levels and that the severity of DFU is positively correlated with the MMP-9 level. Several studies have shown that Sp1 in keratinocytes can directly bind to the MMP-9 promoter and enhance its expression; however, Sp1 is regulated by miR-129 and miR-335. miR-129 or miR-335 can inhibit the expression of MMP-9 by a targeted reduction of Sp1, thus increasing keratinocyte migration, promoting re-epithelialization, and restoring skin thickness and collagen content.[60] That is to say, miRNA activates the function of keratinocytes through targeted regulation of MMP and promotes the re-epithelialization of wounds.

In contrast, other miRNAs inhibit the function of keratinocytes and hinder the re-epithelialization of wounds. miR-203 is the most abundant keratinocyte-specific miRNA in the epidermis and plays an important role in cell differentiation and proliferation. Compared with normal skin tissues, the expression level of miR-203 in patients with DFU was significantly higher and was positively correlated with the severity of DFUs.[14] Studies have shown that miR-203 directly targeted and downregulated IL-8, inhibited the epidermal-mesenchymal transition process, and inhibited keratinocyte proliferation and migration, thereby delaying wound healing.[94] Similarly, miR-

155 inhibited the expression of FGF7 in diabetic wounds, hindered the proliferation and migration of keratinocytes, and delayed the re-epithelialization of wounds.[57] Additionally, studies have shown that miR-210 inhibited its target gene, *E2F3*, which is a cell cycle regulator that promotes the G1/S transition, thereby inhibiting keratinocyte proliferation and re-epithelialization. In contrast, anti-miR-210 increased the proliferation and migration of keratinocytes and significantly accelerated wound healing in diabetic mice.[83]

Regulation of ECM remodeling

ECM remodeling is the last stage of wound healing. Compared with non-diabetic skin, both mouse and patients with diabetes skin are biomechanically inferior, with reduced elasticity, decreased maximum stress, decreased collagen content, and a distortion of ECM production and degradation, in which miRNA has a significant influence.[59,104] Recent studies have shown that MSC treatment can improve the biomechanical properties of damaged diabetic skin by reducing ECM proteolysis. MSC treatment promoted wound healing by upregulating miR-29b, which inhibited the expression of MMP-9 and increased the expression of type I collagen.[62] On the contrary, MSCs downregulated the expression of miR-29a, thereby increasing collagen content and correcting the biomechanical properties of diabetic skin.[59] Another study reported that small EVs produced by the

Table 2. Functions and mechanisms of lncRNAs in diabetic wounds

Regulatory effect	lncRNA	Signaling pathways	Expression change	Function	Experimental model	Mechanism of changing ncRNA	Reference
Anti-inflammatory	lncRNA Lethe	p65-NF-κB	↓	inhibits activation of the p65-NF-κB inflammation signaling pathway and reduces NOX2 expression, thereby reducing macrophage ROS production	type 2 diabetic mice (db/db)	plasmid transfection	36
	lncRNA WAKMAR2	TGF-β-WAKMAR2-p65-NF-κB	↓	inhibits the production of inflammatory chemokines by keratinocytes, while promoting cell migration and accelerating wound re-epithelialization	examined human chronic wounds + in vitro	CRISPR-Cas9 gene-editing technology	37
	lncRNA-ENST00000411554	lncRNA-ENST00000411554/MAPK1	↓	inhibits activation of the MAPK signaling pathway, reduces the production of inflammatory factors, and may mediate cellular immune dysfunction in DFU	examined DFU tissue specimen	—	33
Pro-inflammatory	lncRNA-Gas5	lncRNA-Gas5/STAT1	↑	activates the expression of STAT1 to promote the polarization of macrophages to the M1 phenotype and inhibits wound repair	examined human diabetic skin + in vitro	plasmid transfection	107
	lncRNA MALAT	lncRNA MALAT1/SAA3	↑	regulates the upregulation of glucose-induced inflammatory mediators IL-6 and TNF-α by activating SAA3 to play a pro-inflammatory role	in vitro	siRNA transfection	52
	lncRNA-E330013P06	—	↑	induces inflammatory genes, enhances the response to inflammatory signals, and increases foam cell formation	type 2 diabetic mice (db/db)	lentiviral transfection	108
	lncRNA Dnm3os	NF-κB/lncRNA Dnm3os/TLR4	↑	NF-κB activates the lncRNA Dnm3os promoter, and lncRNA Dnm3os located in macrophages promotes the expression of inflammatory genes by recruiting histone acetyl transferases (HATs) to initiate the transcription of inflammatory factors.	type 2 diabetic mice (db/db)	plasmid transfection	28
Promotes angiogenesis	lncRNA-H19	lncRNA-H19/AKT	↓	promotes angiogenesis by activating the AKT signaling pathway and reverses the inhibitory effect of HG on angiogenesis	type 1 diabetic rats	lentiviral transfection	34
	lncRNA-MALAT1	lncRNA-MALAT1/HIF-1α and VEGF	↓	induces the expression of angiogenic factors and stimulates angiogenesis	examined DFU tissue specimen + in vitro	antisense oligonucleotide	109
	lncRNA-MALAT1	MALAT1/miR-205-5p/VEGF	—	can be used as a molecular sponge of miR-205-5p to	in vitro	lentiviral transfection	32

(Continued on next page)

Table 2. Continued

Regulatory effect	lncRNA	Signaling pathways	Expression change	Function	Experimental model	Mechanism of changing ncRNA	Reference
				promote angiogenesis to enhance the therapeutic effect of MSCs on DFU			
	lncRNA-Leene	–	–	promotes the recruitment of RNA Pol II on the eNOS promoter, enhances the transcription of newborn RNA of eNOS, and promotes angiogenesis	*in vitro*	–	29
Anti-angiogenesis	lncRNA IGF2AS	IGF2AS-IGF2-VEGF	↑	inhibits angiogenesis by inhibiting the expression of angiogenic factors IGF2 and VEGF	*in vitro*	siRNA transfection	110
	lncRNA-H19	lncRNA-H19/miR-152-3p/PTEN/PI3K/AKT	↓	acts as a competitive endogenous RNA to inhibit the miR-152-3p/PTEN/PI3K/AKT signal axis, thereby promoting the proliferation and migration of fibroblasts and inhibiting cell apoptosis	examined DFU tissue specimen + type 1 diabetic mice	plasmid transfection	30
	lncRNA-H19	lncRNA-H19/HIF-1α	↓	recruits EZH_2 to mediate histone methylation, regulates the HIF-1α signaling pathway, promotes fibroblast activation, and accelerates wound repair	*in vitro*	lentiviral transfection	16
Promotes remodeling of the ECM	lncRNA antisense noncoding RNA in the INK4 locus	Antisense noncoding RNA in the INK4 locus/miR-181a/Prox1	–	regulates EMT and the expression of angiogenic factors, inhibits the apoptosis of lymphatic vascular ECs, and increases their migration and tube-like formation to ultimately promote the formation of lymphatic vessels, thereby accelerating the healing of diabetic wounds	type 1 diabetic mice	plasmid transfection	31
	lnc-RNA-Gas5	–	–	inhibits the expression of the transcription factor c-Myc and promotes re-epithelialization to accelerate wound healing	type 1 diabetic mice	–	8
	lncRNA MALAT	lncRNA MALAT/HIF-1α	↑	promotes the activation of fibroblasts in diabetic mice and increases the expression of wound collagen, thereby promoting diabetic wound healing	human *ex vivo* wound models + pigs	lentiviral transfection	35

pretreatment of HUVECs with AGEs (AGEs-sEVs) inhibited collagen synthesis by activating the autophagy of human skin fibroblasts, thus delaying the wound-healing process in rats. Additionally, miR-106b-5p, a protein enriched in fibroblasts, was upregulated in AGEs-sEVs. miR-106b-5p upregulation by fibroblasts can reduce the expression of ERK1/2, leading to fibroblast autophagy and subsequent collagen degradation.[58] Similarly, miR-27-3p inhibited fibroblast function by targeting NOVA1, which resulted in a slower rate of wound healing.[65] Contrarily, anti-miR-378a enhanced the wound-healing process by upregulating synthin-3, which promotes angiogenesis, and vimentin, which promotes the migration and proliferation of fibroblasts.[96] These miRNAs regulate the synthesis and degradation of collagen by

regulating the expression of MMP and can also regulate the function of fibroblasts to play a role in ECM remodeling.

miR-21 is an miRNA with multiple functions, including the regulation of inflammation, angiogenesis, and ECM remodeling during wound healing. miR-21 regulates ECM remodeling by regulating fibroblast proliferation and migration. The expression of miR-21 increases in the late healing process of normal skin wounds but decreases during the healing process of diabetic wounds.[77,105] miR-21 may regulate fibroblast function through the transforming growth factor (TGF)-β1/NF-κB/miR-21 signaling pathway and play a key role in the healing process of diabetic ulcers.[20] In addition, miR-21-3p can also enhance the function of fibroblasts by reducing the expression of its target gene, SPRY1.[64] Previous studies have found that microvesicles (MVs) extracted from transfected keratinocytes are rich in miR-21, and direct administration of MVmiR-21 significantly promoted the healing of skin wounds in diabetic rats. In-depth studies have found that MVmiR-21 promoted the proliferation, migration, and differentiation of fibroblasts; induced the pro-angiogenesis process of endothelial cells; and mediated pro-inflammatory responses. Also, MVmiR-21 increased MMP-1 and MMP-3 levels to regulate the migration of fibroblasts by inhibiting tissue inhibitor of metalloproteinases (TIMPs) and upregulating inflammatory factors to enhance the immune response. MVmiR-21 can also downregulate the protein levels of PTEN and reversion-inducing-cysteine-rich protein with Kazal motifs, activate the MAPK/ERK signaling cascade, and induce expression of α-smooth muscle actin (α-SMA) and N-cadherin, which promotes the differentiation of fibroblasts into myofibroblasts.[95] miR-21-5p can also regulate keratinocyte function by activating the Wnt/β-catenin/MMP-7 signaling pathway.[56]

THE REGULATORY ROLE OF lncRNA IN DIABETIC WOUND HEALING

lncRNAs are a class of ncRNAs longer than 200 nucleotides, which can regulate gene expression at multiple levels including epigenetic regulation, transcription regulation, and post-transcriptional regulation.[27,106] New evidence shows that lncRNAs have significant expression differences in patients with DFUs and affect wound repair (Table 2).

lncRNA regulation of wound inflammation

lncRNAs can play a regulatory role in OS and wound inflammation by controlling the functions of macrophages and T cells. Macrophages polarize from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype during wound healing. Persistent M1 macrophages may cause chronic inflammation in diabetic wounds.[69] The expression of some pro-inflammatory lncRNAs can be induced by hyperglycemia in diabetes mellitus, including lncRNA E330013P06, lncRNA-Gas5, and lncRNA Dnm3os.[28,107,108] Because of the regulatory activities of these lncRNAs, the pro-inflammatory M1 macrophages increased, and the anti-inflammatory M2 macrophages decreased, leading to aggravation of wound inflammation. lncRNA E330013P06 was also upregulated in macrophages of diabetic mice and amplified with increased expression of inflammatory genes. Small interfering (si)RNA-mediated E330013P06 gene silencing inhibited the expression of inflammatory genes induced

by diabetes stimulation, thereby exerting an anti-inflammatory effect.[108] Similar studies have found that lncRNA-Gas5 induced expression of the downstream target gene STAT1, promoted the polarization of macrophages to the pro-inflammatory M1 phenotype, and inhibited the healing of diabetic wounds.[107] Furthermore, studies have shown that NF-κB activation induced an increase in the expression of lncRNA Dnm3os under diabetic conditions, aggravating the inflammatory response. RNA fluorescence in situ hybridization showed that lncRNA Dnm3os in macrophages was localized in the nucleus. Overexpression of Dnm3os in macrophages changed the overall modification of histones, upregulated inflammation and immune response genes, and increased phagocytosis. In contrast, RNAi-mediated lncRNA Dnm3os gene silencing attenuated these responses. Additionally, decreased nucleolin levels and increased lncRNA Dnm3os during diabetes facilitated H3K9ac. It may be that the recruitment of histone acetyltransferase resulted in chromatin relaxation, leading to the upregulation of inflammatory target genes and macrophage dysfunction.[28] Hence, lncRNA acts on macrophages to regulate their phenotypic conversion and the secretion of inflammatory factors to regulate wound inflammation.

T lymphocytes are the key immune cells involved in wound healing. Their activity is often impaired in diabetic wounds, leading to unbalanced inflammatory reactions. Studies have shown that lncRNA-ENST00000411554 regulated the expression of inflammatory factors in T cell-induced responses by downregulating the downstream target gene MAPK1. However, lncRNA-ENST00000411554 is downregulated in DFU patients, mediating the immune-regulation imbalance of these patients.[33] Additionally, the study reported that the level of lncRNA MALAT1 increased early under the HG level but subsequently decreased, which in turn positively upregulated inflammatory ligands such as SAA3 and ultimately increased the production of inflammatory cytokines such as IL-6 and TNF-α. si-MALAT1 can inhibit ROS production in the early stage of OS in endothelial cells.[52] Therefore, the expression of lncRNA in the wound is abnormal, leading to dysfunction of T lymphocytes and abnormal inflammation of the wound.

In addition, lncRNA exerts anti-inflammatory effects by inhibiting the activation of inflammatory signaling pathways. In diabetic wounds, the expression of cellular OS-related genes, ROS and NOX2, was upregulated, whereas anti-inflammatory lncRNA Lethe was downregulated. The overexpression of lncRNA Lethe significantly reduces the translocation of p65-NF-κB to the nucleus and reduces expression of NOX2 and the production of ROS, thereby reducing OS and inflammation and accelerating the healing of diabetic wounds.[36] Similarly, WAKMAR2 inhibits the production of inflammatory chemokines in keratinocytes by regulating the TGF-β-WAKMAR2-p65-NF-κB signaling axis. Studies have found that the expression of anti-inflammatory WAKMAR2 is reduced in diabetic wounds, and its expression is induced by TGF-β signals. Knockout of the WAKMAR2 gene can damage the re-epithelialization of human wounds in vitro and increase the level of inflammation; on the contrary, overexpression of WAKMAR2 inhibits the

Table 3. Regulatory role of circRNAs in wound repair

circRNA	Expression change	miRNA	mRNA	Function	Experimental model	Mechanism of changing the ncRNA	Reference
mmu_circ_0000250	↓	miR-128-3p	sirt1	activates autophagy to inhibit cell apoptosis and promotes the healing of diabetic wounds	type 1 diabetic mice	liposome transfection	39
circ-Amotl1	−	miR-17	STAT3	STAT3 nuclear translocation is promoted and binds to the *DNMT3A* promoter, thereby enhancing DNMT3A expression and regulating miR-17 function.	miR-17 transgenic mice	plasmid transfection	41
hsa_circ_0084443	↑	−	−	inhibits heparin-binding-epidermal growth factor (HB-EGF) and HIF-α to inhibit the migration of keratinocytes and promote cell proliferation	*in vitro*	plasmid transfection	9
circ_0075932	↑	−	PUM2	activates the AuroraA/NF-κB signal axis and plays a pro-inflammatory and pro-apoptotic role in epidermal keratinocytes	burned skin tissues + *in vitro*	lentiviral transfection	43
circ-HIPK3	↓	miR-124	SphK1 STAT3	accelerates the apoptosis and death of ECs	*in vitro*	lentiviral transfection	17

production of inflammatory chemokines by keratinocytes and promotes cell migration.[37]

lncRNA regulates angiogenesis

lncRNAs play a role in promoting angiogenesis in diabetic wound repair through regulation of signaling molecules. lncRNA-H19, in which its expression level is significantly reduced in diabetics, is an angiogenesis-promoting lncRNA. It reverses the inhibition of angiogenesis in diabetics by activating the PI3K-Akt pathway. Tao et al.[34] used high-yield EV-like nanoparticles as an effective lncRNA nano-drug delivery system combined with sodium alginate as a carrier to deliver lncRNA-H19 to wounds, and they observed that lncRNA-H19 significantly promoted the regeneration of blood vessels and significantly accelerated the healing of chronic wounds. It has been reported that Nrf2 and HIF-1α can regulate angiogenesis in DFUs by activating MALAT1/HIF-1α signaling.[109] Expression of lncRNA-MALAT1 was significantly reduced in patients with DFUs, and its reduction was positively correlated with the expression of angiogenic factors Nrf2, HIF-1α, and VEGF. Similar results were observed after knocking out lncRNA-MALAT1. This suggests that Nrf2 can regulate the MALAT1/HIF-1 loop through a positive-feedback mechanism, which may regulate angiogenesis in diabetic wounds. eNOS is a key regulator of endothelial homeostasis and vascular function. Previous studies have shown that lncRNA-Leene can affect endothelial cell function through epigenetic regulation of eNOS expression. In detail, lncRNA-Leene enhancers are located near the *eNOS* promoters, and lncRNA-Leene promotes the recruitment of RNA Pol II to these promoters to enhance eNOS expression, whereas the inhibition of lncRNA-Leene inhibited the expression of eNOS.[29]

In addition, lncRNAs have anti-angiogenic effects. IGF2 is a key transcription factor that regulates cardiovascular function in patients with diabetes, and its antisense nucleic acid IGF2AS is an lncRNA. Research has shown that the expression of IGF2ASII in myocardial microvascular endothelial cells was upregulated, whereas the expression of IGF2 was downregulated. The inhibition of IGF2AS can upregulate IGF2 and VEGF, enhance the proliferation and invasive ability of endothelial cells, and promote angiogenesis.[110]

lncRNA regulates ECM remodeling

The interaction and abnormal expression of lncRNAs and miRNAs may explain inhibition of ECM remodeling in diabetic wounds. Previous studies have confirmed that the lncRNA-H19/miR-152-3p/PTEN axis can regulate the biological activity and inflammatory response of fibroblasts and influence healing of DFUs. When MSC-exos overexpressing lncRNA-H19 were co-cultured with fibroblasts, their proliferation and migration significantly improved, apoptosis and inflammation were inhibited, and lncRNA-H19 significantly facilitated wound healing in DFU mice.[30] In addition, H19 can recruit enhancer of zeste (EZH) 2 to mediate HIF-1 histone H3K4me3 methylation, increase the expression of HIF-1α, and enhance the function of fibroblasts.[16] Similarly, lncRNA-MALAT1 facilitated the activation of fibroblasts in diabetic mice by activating the HIF-1α signaling pathway, which increased wound collagen synthesis and facilitated healing.[35]

REGULATORY ROLE OF circRNAs IN DIABETIC WOUNDS

Wang et al.[9] reported that 115 circRNAs were upregulated and 111 circRNAs were downregulated in DFUs, suggesting that circRNAs may be involved in the repair process, including cell proliferation and migration, apoptosis and autophagy, and angiogenesis (Table 3).[9,17,41,42,46]

It was reported that the circRNA expression level in HUVECs could be altered by HG levels. Sequencing results showed 214 differentially expressed circRNAs, among which three showed the highest upregulation (hsa_circ_0008360, hsa_circ_0000109, and hsa_circ_0002317). Bioinformatics analysis indicated that these circRNAs regulate the

Table 4. Regulatory effects of different ncRNAs on key genes and signal pathways

Target	ncRNA	Function
VEGF	miR-15b, miR-23, miR-135a-3p, miR-200b miR-205-5p, miR-210, miR-217, miR-615-5p, miR-1248, lncRNA-MALAT1, lncRNA IGF2AS	angiogenesis collagen synthesis, etc.
HIF-1α	miR-15b, miR-23c, miR-210, miR-217, miR-1248, lncRNA-MALAT1, lncRNA-H19 hsa_circ_0084443	angiogenesis re-epithelialization collagen synthesis, etc.
ROS	miR-21, miR-27b, miR-34a, miR-126, miR-155, miR-5591-5p, lncRNA-MALAT1, lncRNA Lethe	inflammation
MMP-9	miR-29b, miR-129, miR-217, miR-335	ECM remodeling
Signal pathways		
NF-κB	miR-21, miR-132, miR-146a, miR-155, miR-let-7d, lncRNA Lethe, lncRNA WAKMAR2, lncRNA Dnm3os circ_0075932	inflammation
PI3K/AKT	miR-21, miR-126-3p, miR-203, lncRNA-H19	cell proliferation
MAPK	miR-21, miR-126, miR-203, miR-135a-3p, miR-5591-5p, lncRNA-MALAT1, lncRNA-ENST00000411554	cell proliferation
Wnt	miR-21, miR-20b-5-p	cell proliferation

expression of vascular endothelial function and angiogenesis-related genes by targeting miRNAs.[46] HG levels trigger the regulation of endothelial cell damage through the circHIPK3/miR-124/SphK1/STAT3 axis. In other words, overexpression of circHIPK3 can inhibit HG-induced cell death and apoptosis; contrarily, targeted siRNA silencing of circHIPK3 caused the death of endothelial cells. Further studies have found that HG-induced downregulation of circHIPK3 may lead to the accumulation of miR-124 in HUVECs and human arterial endothelial cells (HAECs), whereas miR-124 inhibits endothelial cell activity, promotes apoptosis, and impairs endothelial cell migration and tube formation.[17] Additionally, studies have shown that ADSC-exo containing mmu_circ_0000250 promoted the expression of its downstream gene *SIRT1* by adsorbing miR-128-3p facilitating the activation of autophagy under HG conditions. *In vivo* experiments showed that exos containing high concentrations of mmu_circ_0000250 accelerated the healing of diabetic wounds. Histopathological examinations show that it inhibited cell apoptosis through the activation of autophagy and facilitated angiogenesis in the skin wound.[39]

circRNAs also play an important role in wound healing by regulating the functions of keratinocytes and fibroblasts. Wang et al.[9] showed that the circRNA hsa_circ_0084443 upregulated keratinocyte migration and proliferation in DFUs. Zhang et al.[43] found that circ_0075932 in ADSC-EXO induced inflammation and apoptosis

of human dermal keratinocytes by directly binding to PUM2 and promoting PUM2-mediated AuroraA/NF-κB pathway activation. Additionally, Yang et al.[41] reported that circRNAmotl1 encouraged wound repair by promoting fibroblast proliferation, survival, adhesion, and migration. Research has shown that circRNAmotl1 carries out the above-mentioned activities by promoting the nuclear translocation of STAT3, which binds the DNMT3A promoter to facilitate its transcription and translation. DNMT3A induces the methylation of the miR-17 promoter and reduces the expression of miR-17-5p (Table 4).

THE APPLICATION PROSPECTS OF ncRNA IN DIAGNOSIS AND TREATMENT

ncRNAs often respond differently in various pathological conditions, and changes in their expression directly reflect the physiological state of cells. Moreover, differences in the expression of ncRNA have been reported in both type 1 diabetes and T2D of animal studies,[51,53,71,73] indicating that ncRNA influences both types of diabetic wounds; however, whether insulin resistance is a differentiating factor in T2D wounds remains unknown. miRNA expression profile analysis of fibroblasts extracted from DFUs showed significant distortion that affected important stages of wound repair.[67,111,112] Another study identified 58 upregulated lncRNAs and 42 downregulated lncRNAs in DFUs compared with non-patients with diabetes.[113] Similarly, HG induced changes in the expression of lncRNAs and related mRNAs.[114] Additionally, there were significant differences in the expression of circRNAs in DFUs,[9] and the expression profile of circRNAs in HUVECs induced by HG was also significantly different. Further bioinformatics analysis showed that these circRNAs regulated the expression of vascular endothelial function and angiogenesis-related genes by targeting miRNAs.[46] Therefore, we can diagnose and treat diseases by detecting the content of specific ncRNAs.

ncRNA as a diagnostic biomarker

Previous studies have confirmed that ncRNAs are differentially expressed in patients with diabetes and are closely related to the occurrence and development of the disease.[113,115,116] Lin et al.[88] showed that the serum levels of miR-217 in DFU patients was significantly upregulated compared with those of healthy individuals. Moreover, as the Wagner grade increases, the serum miR-217 level gradually increases. In other words, the expression level of mir-217 is positively correlated with the severity of DFUs. Another study also found that compared with normal skin tissue, the expression level of miR-203 in the skin of DFUs was significantly higher, and the expression profile of miR-203 was positively correlated with the severity of DFUs. Moreover, compared with other parameters used to assess the severity of DFUs, the measurement of miR-203 is more accurate and effective.[14] In addition, studies have found that baseline serum miR-15a and miR-16 levels were positively correlated with restenosis after amputation in T2D patients.[117] Therefore, the level of expression of ncRNA may provide a new reference index for the clinical classification DFUs.

In addition, the physiological and pathological changes of the human body may be reflected by the changes in the expression of ncRNA in

the blood. For example, circulating exosomal miR-20b-5p has been proven to be an effective and non-invasive biomarker for the early diagnosis of non-small cell lung cancer and nasopharyngeal carcinomas.[118,119] The correlation between miR-20b-5p and diabetes is not yet clear. Further studies have found that miR-20b-5p from circulating exos in patients with diabetes was significantly upregulated, and such exosomal intervention may hinder wound healing.[103] Similarly, Dangwal et al.[90] reported reduced levels of miR-191 and miR-200b in blood in T2D patients compared with healthy individuals. Patients with diabetes subjects with associated peripheral artery disease and chronic wounds showed higher levels of circulating C-reactive protein and pro-inflammatory cytokines. These results indicate that monitoring the expression of ncRNAs may be adopted as an accurate and effective biomarker for the clinical evaluation of the severity of diabetic wounds.

ncRNA can be used as a target for wound-repair treatment

Recent studies have shown that ncRNAs play important roles in cancer and cardiovascular disease and have indicated most drugs currently have potential ncRNA binding sites.[120] This provides a basis for the development of drugs that use ncRNAs as a therapeutic target. For example, ginsenoside uses miR-23a as a drug target. Downregulating the expression of miR-23a reduced its inhibitory effect on downstream gene *IRF-1* and upregulated iNOS, promoted wound angiogenesis, and facilitated the healing of DFUs.[78] Sawaya et al.[8] reported that the topical application of mevastatin accelerated wound healing; one of the possible mechanisms is that mevastatin induced the expression of lncRNA-Gas5. lncRNA-Gas5 inhibits the expression of the transcription factor c-Myc, which regulates the proliferation and migration of keratinocytes, thereby promoting wound healing.

Inhibition of the healing process in diabetic wounds may partly be due to abnormal expression of ncRNAs. The restoration of the expression level by exogenous supplementation or inhibition of the related ncRNAs may be an ideal method to speed healing of diabetic wounds. miR-21 expression is significantly reduced in diabetic wounds. Lv et al.[56] loaded exogenous miR-21-5p into hADSC-exos using electroporation and significantly accelerated wound healing. Similarly, Tao et al.[34] delivered lncRNA-H19 to wounds using extracellular-simulated vesicle nanocapsules as the delivery system and observed a significant stimulation of angiogenesis. In addition, miR-146a, which possess anti-inflammatory properties, was downregulated in diabetic wounds. Zgheib et al.[74] used cerium oxide nanoparticles to deliver miR-146a to diabetic mice skin wounds, which improved wound inflammation, increased angiogenesis, and significantly accelerated wound closure.

In addition to restoring the expression level of ncRNAs in wounds through a delivery system, it has also been shown that damage resulting from abnormal ncRNA expression can be reversed by directly using antisense ncRNAs or antagonists. MRG-110, a synthetic antiangiogenic molecule, inhibited the expression of miR-92a and accelerated angiogenesis and the healing of both diabetic and non-diabetic wounds.[81] Similarly, the direct use of light-induced anti-miR-92a also promoted skin repair in healing impaired diabetic mice.[82] Direct application of miR-155 inhibitors to wounds also reduced inflammation, promoted cell migration, and shortened diabetic wound closure time.[47,57] Furthermore, Pizzino et al.[92] injected antagomir-15b and -200b into the wound edge of the back skin of diabetic mice, which changed the expression level of downstream angiogenesis genes and improved the healing outcome.

Finally, ncRNAs can also be used as therapeutic targets for stem cell therapy to regulate the healing process of diabetic wounds. MSC treatment of diabetic wounds is mainly through the upregulation of the expression level of miR-146a, which is involved in the regulation of immune and inflammatory responses, resulting in downregulation of downstream inflammatory target genes.[73] MSCs can also reduce the level of miR-29a, increase the content of collagen, and correct the damaged biomechanical properties of patients with diabetes skin.[59] Similarly, MSCs can inhibit the expression of MMP-9 through the action of miR-29b and increase the expression of type I collagen, promoting wound healing.[62]

Challenges in the application of ncRNAs

Since ncRNAs have different effects in different stages of diabetic wound healing by regulating gene expression at the molecular level, they have broad application prospects in diabetic wound diagnosis and treatment. However, the current research on ncRNAs has just started, especially lncRNAs and circRNAs, still facing some of the following challenges, hence further research into their clinical application is needed.

First, use of ncRNAs as diagnostic markers due to their insufficient specificity, low sensitivity, and individual differences further limits application as therapeutic targets. ncRNAs may participate in several important processes of wound healing; however, their role may differ at each stage. ncRNAs are dynamic and unstable, differ largely in their mode of action, and possess limited specificity. For example, miR-21 is significantly upregulated during M1 polarization of macrophages, and consequent upregulation of inflammation-related factors exerts a pro-inflammatory effect.[77,95] The expression of miR-21 decreases in the mid-stage of wound healing, which weakens its pro-inflammatory effect and exerts an anti-inflammatory effect; however, it is upregulated in the later stage of wound healing to facilitate the proliferation and migration of keratinocytes and fibroblasts. miR-21 can also target and regulate the expression of downstream genes *MMP-1*, *MMP-3*, *TIMP3*, and *TIMP4*; regulate the remodeling and degradation of ECM; and promote re-epithelialization[56,77,95,98] and can also regulate the transdifferentiation of cells in wound healing.[76] Furthermore, the levels of various ncRNAs are differently affected by the expression level of cytokines or inflammatory factors in the wound, which cause individual differences. For example, TGF-β1 can induce the expression of miR-21 and WAKMAR2,[20,37] and the expression levels of miR-191 and miR-200b are significantly correlated with IL-1β, platelet-derived growth factor (PDGF)-BB, macrophage migration inhibitory factor (MIF), and C-reactive protein.[90] Moreover, TNF-α can also induce an

increase in the expression of miR-200b during chronic inflammation in diabetes.[61] In other words, differences in the level of inflammation in each patient will also lead to differences in the activities of ncRNAs.

On the other hand, in addition to the dynamic and individual differences, the activities of ncRNAs may overlap, and their interactions are complex; that is, ncRNAs have multiple downstream target genes, regulate different signaling pathways, and participate in different physiological processes of various cells and tissues, so their actions differ across cells, tissues, and target sites.[16,30,48,75,121] A disease may be accompanied by several changes in the expression of different ncRNAs,[111,112,122] and their functions differ markedly.[84] The expression dynamics and individual differences of ncRNAs as well as their mutual relationship are like a complex network, which greatly increases the challenge of clinical application of ncRNAs. Use of ncRNAs as a diagnostic biomarker is based on changes in their level of expression or restoration of expression through artificial supplementation for treatment purposes. There is a need for a patient-based approach; that is, more information about the patient is needed for the successful application of ncRNAs in disease diagnosis and treatment. In addition, because each ncRNA can regulate different signaling pathways and play different roles in different tissues and cells, research regarding the clinical effectiveness of ncRNAs is lacking. Researchers must also ascertain whether reversing expression levels will have serious side effects, due to individual differences in ncRNAs expression. Moreover, the statistical methods used in various studies may differ. Therefore, an objective and rational comprehensive analysis of the expression differences of these ncRNAs is warranted.

The use of ncRNAs as a target for diabetic wound treatment requires choosing appropriate methods to synthesize the necessary ncRNA mimics or inhibitors, but we currently lack reliable gene-modification technology. There are two treatment strategies: (1) use of ncRNA mimics or viral vectors to trigger the overexpression of previously inhibited ncRNAs or (2) use of chemically modified antisense ncRNA oligonucleotides to inhibit the expression of previously overexpressed ncRNAs.[47,71,72,80] Plasmids are commonly used to construct overexpression vectors, and lentiviral transfection techniques are used to synthesize ncRNA. These techniques are not suitable for clinical treatment due to biological safety concerns,[123] which greatly limits their clinical application.

The clinical application of ncRNAs must depend on a safe, efficient, and reliable delivery system, which we currently lack. ncRNA use mainly includes the direct application of synthetic inhibitors or mimics of corresponding ncRNAs. However, ncRNAs are very easily hydrolyzed in the microenvironment of wounds, and it is difficult for free ncRNAs to enter the cell membrane, resulting in little or no effect.[56] Existing studies have shown that exos are also promising new drug-delivery vehicles because they can protect their payload from chemical and enzymatic degradation and can evade recognition and subsequent elimination by the immune system.[103] Several studies have used exo carriers to deliver ncRNAs to wound sites, which can restore the expression level of ncRNAs in the body, regulate immune responses,

inhibit inflammation, and promote cell proliferation and angiogenesis. The combination of ncRNAs and exos significantly facilitates and improves the quality of wound healing.[30,55,56,98] However, exos have disadvantages, such as complex extraction, low yield, and high cost, hindering clinical application.[124,125] Moreover, there is a lack of appropriate technology for the transfer of ncRNAs into exos.

Furthermore, the current research on ncRNAs and their possible application in the treatment of DFUs is basic, and clinical research is still lacking. Moreover, the models used in basic research possess certain limitations. Existing *in vivo* studies are mainly restricted to mouse and rat wound models. Wound healing in rats is mainly completed by skin contraction, whereas in humans, it involves granulation tissue repair; thus, the rat model cannot simulate the physiological repair process of human skin defects. In addition, the existing diabetic wound model is mainly an acute wound model, which cannot simulate the chronic state of complications caused by long-term hyperglycemia. The use of diabetic pigs as a model in the studies of wound healing is an ideal substitute because their skin is structurally similar to human skin.[8,74]

Conclusions

ncRNAs, especially miRNAs, are involved in the wound-healing process, including the regulation of inflammation, granulation tissue formation, re-epithelialization, and ECM remodeling. With the advancement in technology and research, ncRNAs are expected to become a new, accurate, and effective biomarker for disease diagnosis and potential therapeutic targets. However, current research on ncRNAs is still in its infancy, especially lncRNA and circRNAs. Moreover, most of these studies are mainly basic research; hence, preclinical and clinical studies are required in order to verify clinical applications. More specific ncRNAs should be screened as diagnostic markers and therapeutic targets. Research and development of more efficient and convenient ncRNA delivery systems are also warranted.

AUTHOR CONTRIBUTIONS
C.D., G.X., F.Q., and J.L. designed the concept. J.L. and M.W. searched the literature and wrote the manuscript. X.L., S.X., and Y.C. created the figures. F.L. and J.T. created the tables. C.D., G.X., and F.Q. revised the manuscript. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS
The authors declare no competing interests.

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