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Long non-coding RNAs in diabetic wound healing: Current research and clinical relevance

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Abstract

Diabetic wounds are a protracted complication of diabetes mainly characterised by chronic inflammation, obstruction of epithelialization, damaged blood vessels and collagen production (maturation), as well as neuropathy. As a non-coding RNA (ncRNA) that lack coding potential, long non-coding RNAs (lncRNAs) have recently been reported to play a salient role in diabetic wound healing. Here, this review summarises the roles of lncRNAs in the pathology and treatments of diabetic wounds, providing references for its potential clinical diagnostic criteria or therapeutic targets in the future.

KEYWORDS
diabetes, long non-coding RNA, wound healing

Abbreviations: AGEs, advanced glycation end products; ANRIL, antisense RNA to INK4 locus; ASOs, antisense oligonucleotides; bFGF, basic fibroblast growth factor; ceRNA, competing endogenous RNA; CHR, cell cycle genes homology region; COL, collagen; CTGF, connective tissue growth factor; DFUs, diabetic foot ulcers; DM, diabetic mellitus; DPN, diabetic peripheral neuropathy; DRG, dorsal root ganglion; DSG1, desmoglein1; EMNVs, extracellular vesicle-mimetic nanovesicles; EZH2, enhancer of zeste homologue 2; FAP, fibroblast activation-related protein; FBN1, fibrillin 1; FLG, filaggrin; GAS5, growth arrest-specific 5; HBEGF, heparin binding EGF like growth factor; HEKn, neonatal epidermal keratinocytes; HG, high glucose; HIF-1α, hypoxia induced factor-1α; HMECs, human skin microvascular endothelial cells; H3K4me3, trimethylation of lysine 4 on histone H3 protein subunit; IGF, insulin-like growth factors; IGF2AS, antisense RNA of IGF2; LECs, lymphatic endothelial cells; LCE1B, late cornified envelope 1B; lncRNAs, long non-coding RNAs; LOR, loricrin; LPS, lipopolysaccharide; MALAT1, metastasis associated lung adenocarcinoma transcript 1; MAP K1, mitogen-activated protein kinase 1; MIAT, myocardial infarction associated transcript; miR, microRNA; MMP, matrix metalloprotein; mMVE, myocardial microvascular endothelial cells; MSC, mesenchymal stem cell; MT1P3, metallothionein 1 pseudogene 3; MWT, mechanical withdrawal threshold; ncRNA, non-coding RNA; NOX, NADP oxidase; PDGF-BB, platelet-derived growth factor; Plod 1, procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1; PPAR, peroxisome proliferator-activated receptor; PRANCR, progenitor renewal-associated non-coding RNA; Prox1, prospero homeobox 1; PTEN, phosphatase and tensin homologue deleted on chromosome 10; PVT1, plasmacytoma variant translocation 1; RIP, RNA binding protein immunoprecipitation; RNAi, RNA interference; RNA-seq, RNA-sequencing; ROS, reactive oxygen species; SAA3, serum amyloid antigen 3; shRNAs, small hairpin RNAs; siRNAs, small interfering RNAs; SNCV, sensory nerve conduction velocity; SRF, serum response factor; STAT1, signal transducer and activator of transcription 1; STZ, streptozocin; TDG, thymine-DNA glycosylase; TET2, ten-eleven translocation 2; TETILA, TET2-interacting long non-coding RNA; TWL, thermal withdrawal latency; VEGFA, vascular endothelial growth factor A.

Le Kuai and Jing-Si Jiang contributed equally to this study.

Key Messages

- Diabetic ulcers are a serious complication of diabetes with substantial morbidity and mortality, and long non-coding RNAs (lncRNAs) have recently been reported to play a salient role in diabetic wound healing
- In this review, the related lncRNAs in the pathology and treatments of diabetic wounds were summarised with an emphasis on their prognostic and therapeutic potential. We clearly separated the findings from cell-cultured studies, animal studies and the studies in humans with evaluation of the strength of the evidence for the role of lncRNAs in each phase of healing (inflammation, reepithelialisation, maturation and neuropathy)
- In the article, we included clear practical messages and discussed the limitation of this approach, providing a reference for its potential clinical diagnostic criteria or therapeutic targets in the future. We believe that our article would make a significant contribution to the literature, because it is the first review about the roles of lncRNAs in diabetic wound healing

1 | INTRODUCTION

Diabetic foot ulcers (DFUs) are chronic and refractory wounds caused by peripheral vascular neuropathy and microcirculatory disorders. The pathological factors of DFUs remain undetermined, which can be roughly divided into chronic inflammation, angiopathy and neuropathy.[1-3] Statistically, the global prevalence rate of diabetes is 9.3% in 2019 and evaluated to be 10.9% by 2045.[4] Furthermore, the lifetime risk of a diabetic individual developing an ulcer reaches 25%, which is the foremost serious complication of diabetes with substantial morbidity and mortality.[5-7] Long non-coding RNAs (lncRNAs) are non-protein coding RNAs with a length of more than 200 nucleotides.[8,9] It participates in physiological and pathological processes by regulating gene expressions.[10-14] Previous research has shown that lncRNAs may be involved in diabetes-related diseases.[15] Studies based on RNA-sequencing (RNA-seq) have identified a multitude of differentially expressed lncRNAs in diabetic wounds compared with non-diabetic wounds.[15-18] In this review, we overview the roles of lncRNAs in diabetic wound healing, focusing on the currently known mechanisms and functions of lncRNAs with particular emphasis on their prognostic and therapeutic potential.

2 | LNCRNAs INVOLVED IN HAEMOSTASIS

Haemostasis represents the initiation of wound healing (0-several hours after injury), during which the clotting factors are immediately released, platelets are activated and aggravated, thus resulting in the formation of blood clot.[19] The abnormalities of platelets in diabetic mellitus (DM) are characterised to be hyperactive with increased autophagy, activation, adhesion and aggregation, causing wound healing dysfunction.[20]

2.1 | The expression patterns of lncRNAs in diabetic patients

Zhou et al[21] conveyed that lncRNA metallothionein 1 pseudogene 3 (MT1P3) and p2y12 receptor (a Gi-coupled receptor predominantly expressed on platelets) were correlatively up-regulated in megakaryocytes from diabetic patients, implying that MT1P3 may be responsible for cutaneous wound healing of diabetes.

2.2 | The expression patterns of lncRNAs in diabetes animal models

In both ob/ob mice and GK rats, the levels of MT1P3 and p2y12 (mRNA and protein) in megakaryocytes were higher than that of C57BL/6J mice. It has been investigated that the activation of p2y12 leads to platelet aggregation.[22] When knocking down the expression of MT1P in GK rats, the mRNA and protein expressions of p2y12 were decreased. These results indicated that MT1P3 positively correlated with p2y12 and aggravate platelet activation and aggregation.[21]

2.3 | The potential molecular mechanisms of lncRNAs

In HG-cultured Dami cells, induced MT1P3 and p2y12, reduced miR-126 (involved in P2Y12 receptor regulation[23]) were observed. Interestingly, it was predicted and

confirmed that MT1P3 bound to miR-126. Therefore, MT1P3 may positively regulate platelets' hyperactivation by up-regulating p2y12 through sponging miR-126.[21]

To conclude, clinical, in vivo and in vitro evidence combined suggested that MT1P3 harassed normal haemostasis in diabetic environment. We implore to further investigate how MT1P3 functions in the haemostasis process of delayed wound healing of diabetes. Moreover, studies concerning other lncRNAs involved in haemostasis phase of diabetic wound healing demand ascertainment (Table 1, Figure 1).

3 | LNCRNAs INVOLVEMENT IN INFLAMMATION

3.1 | The expression patterns of lncRNAs in diabetic patients

Related study[24] showed that the wound tissues of DFU patients were infiltrated with increased neutrophils, lymphocytes and inflammatory cytokines including IL-1β, IL-2, IL-10, IFN-γ and TNF-α. Based on RNA-seq, eight lncRNAs decreased most significantly in DFUs were screened, of which lncRNA-ENST00000411554 was the upstream regulation link of mitogen-activated protein kinase 1 (MAPK1). MAPK1 is an essential pathway involved in the regulation of cutaneous healing in diabetes.[25] It was inferred that lncRNA-ENST00000411554/MAPK1 axis related to excessive inflammatory infiltration in DFUs.

Up-regulated lncRNA-H19 and down-regulated microRNA (miR)-29b were detected in the blood samples of patients with DM. With the increase of blood glucose, the expression of H19 and the protein level of vascular endothelial growth factor A (VEGFA) involved in the early inflammation stage increased, while the expression of miR-29b decreased, indicating the role of H19/miR-29b axis in the inflammatory pathology of DM.[29]

In normal wound healing, the polarisation of M1 macrophages is increased in the early stage of inflammation, releasing early inflammatory cytokines and exacerbating the production of reactive oxygen species (ROS). In the later stage, the transition of M1 macrophages to M2

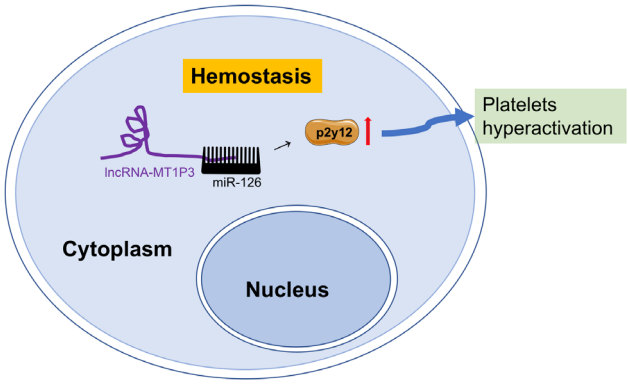

FIGURE 1 LncRNA involved in haemostasis process of diabetic wound healing. LncRNA-MT1P3 binds with miR-126 to up-regulate the expression of p2y12, resulting in platelets hyperactivation by up-regulating

TABLE 1 lncRNAs involvement in haemostasis and inflammation

lncRNA	Expressions	Potential functions	Proposed mechanisms	Clinical relevance	Ref.
MT1P3	Up-regulated in megakaryocytes from diabetic patients	Aggravates platelets activation	Sponges miR-126 to increase p2y12 expression	Biomarker	(21)
ENST00000411554	Down-regulated in the wound tissues of DFUs	Down-regulates inflammation	Restrains MAPK pathway	Biomarker	(24)
H19	Up-regulated in the blood samples of DM	Aggravates inflammation	Sponges miR-29b to up-regulate VEGFA through AKT/eNOS signalling pathway	Biomarker	(25)
GAS5	Up-regulated in M1 macrophages of diabetic wound skin	Promotes the polarisation of M1 macrophages	Activates STAT1 signalling	Biomarker	(26)
MALAT1	Up-regulated in HG-cultured HUVECs	Up-regulates inflammation	Up-regulates IL-6 and TNF-α through activation of SAA3	Unknown	(27)
Lethe	Down-regulated in the wounds of diabetic mice	Inhibits ROS production	Down-regulates NOX2 via NF-κB pathway	Unknown	(28)

Abbreviations: DFUs, diabetic foot ulcers; DM, diabetic mellitus; HG, high glucose; MAPK, mitogen-activated protein kinase; NOX2, NADP oxidase 2; ROS, reactive oxygen species; SAA3, serum amyloid antigen 3; STAT1, signal transducer and activator of transcription 1; VEGFA, vascular endothelial growth factor A.

macrophages is enhanced, facilitating the maturation and healing of wounds.[30,31] Abnormally, the continuous polarisation of M1 macrophages in diabetic patients leads to inflammatory infiltration and delayed wound healing.[32,33] Hu et al[26] observed higher expression of lncRNA-GAS5 (Growth Arrest-Specific 5) in diabetic wound skins than non-diabetic and it was mainly expressed in M1 macrophages. GAS5 up-regulated in macrophages was concerned with chronic inflammation of diabetic ulcers.

3.2 | The expression patterns of lncRNAs in diabetic mice

Consistent with the results in diabetic patients, higher level of GAS5 was found in M1 macrophages of diabetic mice skin, indicating the relation of GAS5 and M1 macrophage polarisation.[26]

On the contrary, lncRNA-Lethe was down-regulated in the wound of diabetic mice (1 and 3 days post-wounding) and correlated with increased gene expression of NADP oxidase (NOX)-2, an enzyme that is highly expressed by inflammatory cells and produces ROS in wounds.[28] Lethe down-regulation might be responsible for excessive ROS production.

3.3 | The potential molecular mechanisms of lncRNAs

Based on the overexpression of H19 in the blood samples of DM patients, Cheng et al[29] further confirmed that H19 knockdown attenuated TNF-α and VEGFA protein expressions, ROS production and NOX activity, while increased miR-29b level and AKT/eNOS activation in HUVECs. Notably, H19 directly bound to miR-29b, while miR-29b specifically inhibits the protein expression of VEGFA, suggesting H19 acted as a microRNA sponge for miR-29b to aggravate oxidative stress and inflammatory infiltration.

Puthanveetil et al[27] found that the mRNA expressions of serum amyloid antigen 3 (SAA3, inflammatory regulatory factor), lncRNA-MALAT1 (metastasis associated lung adenocarcinoma transcript 1), the protein expressions of IL-6 and TNF-α increased in high glucose (HG)-cultured HUVECs, while MALAT1 knockdown reversed these results. Moreover, knocking down SAA3, the protein expressions of IL-6 and TNF-α decreased. Meanwhile, the decreased expressions of IL-6 and TNF-α protein caused by MALAT1 knockdown could be increased by SAA3 overexpression. These results were consistent in the endothelial cells of diabetic mice, suggesting that MALAT1 inhibitors may suppress the expression of SAA3 in diabetic HUVECs and reduce inflammatory infiltration.

Signal transducer and activator of transcription 1 (STAT1) positively regulates the polarisation of M1 macrophages.[34] Interestingly, GAS5, which was highly expressed in the macrophages of Db mice, could optimise the polarisation of M1 macrophages by promoting the mRNA and protein expressions of STAT1.[26] The evidence mentioned above is indicative of the pathological role of GAS5 in diabetic wounds.

In HG-cultured RAW264.7 macrophages and bone marrow-derived macrophages of diabetic mice, the expression of Lethe decreased, while the mRNA levels of NOX2 and the ROS production increased. Overexpression of Lethe resulted in decreased mRNA expression of NOX2 and ROS production. Notably, NOX2 in macrophages is regulated by NF-κB pathway and Lethe reduces the activation of NF-κB pathway by negatively regulating p65-NF-κB binding.[35,36] Lethe may inhibit the activation of NF-κB, reduce the expression of NOX2 and the production of ROS by combining to NF-κB subunit p65.[28]

Taken together, the differential expressions of lncRNA-ENST00000411554, H19 and GAS5 in diabetic patients relate to MAPK signalling, VEGFA release and macrophage phenotype, respectively, showing reliable evidence for these lncRNAs as potential biomarkers for diabetic wounds. Besides, the molecular mechanisms of H19 and GAS5 in inflammation have been shown. However, further studies of lncRNA-ENST00000411554 in DM are imperative for a greater understanding of its role in pathogenesis. Besides, the down-regulation of Lethe in diabetes should be delineated in diabetic patients. In terms of MALAT1, in vivo research studies are needed to confirm its potential role in inflammation of diabetic ulcers (Table 1, Figure 2).

4 | LNCRNAs AND REEPITHELIALISATION

Reepithelialisation including the proliferation and migration of keratinocytes are deteriorated in diabetic wounds. Several lncRNAs including PRANCRA, H19, uc.291 and GAS5 were associated with it.

4.1 | The expression patterns of lncRNAs in diabetic patients

Sawaya et al[37] found that reepithelialisation and wound healing of diabetic patients were accelerated with mevastatin treatment. Furthermore, increased expression of

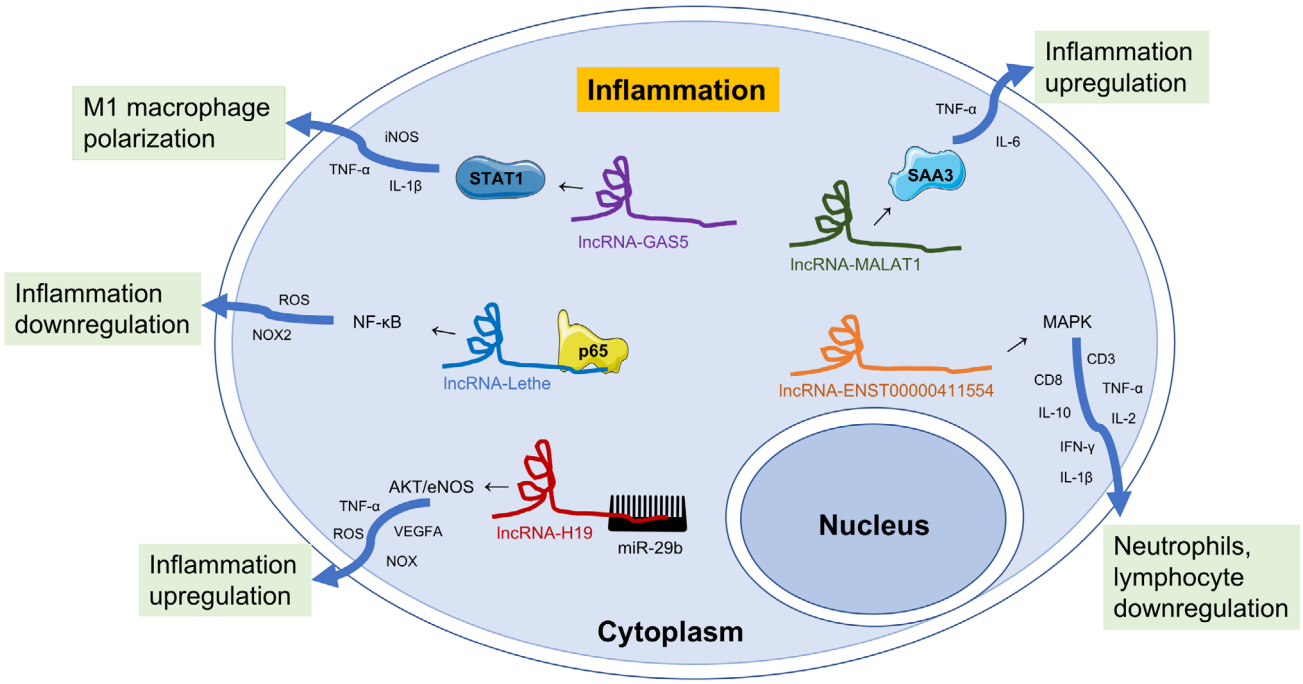

FIGURE 2 Roles of lncRNAs in inflammation of diabetic wound healing. LncRNA-ENST00000411554 down-regulates the protein expressions of CD3, CD8, IL-1β, IL-10, IFN-γ and TNF-α in diabetic wounds by restraining mitogen-activated protein kinase 1 (MAPK) pathway. LncRNA-H19 sponges miR-29b and positively regulates the expressions of vascular endothelial growth factor A (VEGFA), TNF-α, reactive oxygen species (ROS) production and NADP oxidase (NOX) activity through suppressing AKT/eNOS signalling pathway. LncRNA-GAS5 promotes the mRNA expressions of iNOS, IL-1β, and TNF-α by enhancing the mRNA and protein expressions of signal transducer and activator of transcription 1 (STAT1), resulting in increased polarisation of M1 macrophages. LncRNA-MALAT1 stimulates the mRNA and protein expressions of serum amyloid antigen 3 (SAA3), inducing IL-6 and TNF-α. LncRNA-Lethe directly binds with p65 to inhibit the activation of NF-κB, thus down-regulating NOX2 and ROS production

GAS5 was observed in epithelial keratinocytes. Mevastatin treatment may improve diabetic wound healing by up-regulating GAS5 to enhance reepithelialisation.

4.2 | The potential molecular mechanisms of lncRNAs

In primary epidermal keratinocytes of neonatal foreskin, progenitor renewal-associated non-coding RNA (PRANCR) was identified as a highly expressed gene related to epithelial cell cycle regulation. Relatively, proliferation and differentiation of keratinocytes, the gene expressions of transcription factors E2F4 and FOXM1 were down-regulated in keratinocytes of PRANCR knockdown. The levels of E2F target genes, including CCNB1, CCNB2, CDC25C and CDK1, were also reduced.[38] Chen et al[39] implicated that the protein complex of E2F and FOXM1 bound to the cell cycle genes homology region (CHR) sequence of gene promoters, while the CHR sequence of cell cycle-related genes can be inhibited by TP53 through CDKN1A and transcription inhibitory factor DREAM (TP53-CDKN1A-DREAM-CHR) and suppress cell proliferation and differentiation.[40,41] In keratinocytes with PRANCR knockdown, the expression of CDKN1A was increased, impairing cell proliferation and differentiation. These data indicated that PRANCR knockdown may attenuate the transcription of CCNB1, CCNB2, CDC25C and CDK1 through TP53-CDKN1A-DREAM-CHR pathway and restrain regeneration of epithelium.[42]

Interestingly, desmoglein1 (DSG1) promotes the differentiation of keratinocytes through MAPK/ERK pathway.[43,44] Li et al[45] found that miR-130b-3p had a targeted inhibition on DSG1, while H19 may sponge miR-130b-3p and facilitate keratinocyte differentiation as a competing endogenous RNA (ceRNA).

According to RNA-seq analysis, the expression of lncRNA-uc.291 increased during the differentiation of human epithelial keratinocytes. In neonatal epidermal keratinocytes (HEKn), uc.291 knockdown decreased the mRNA and protein expressions of the late-differentiation marker loricrin (LOR) and filaggrin (FLG). ACTL6A, a BAF-related protein (BRM/BRG1, optimising the differentiation of epidermis), was predicted and confirmed to directly bind with uc.291 and it is highly expressed in proliferative epithelial progenitor cells.[46,47] Recently, it

TABLE 2 lncRNAs and reepithelialisation

lncRNA	Expressions	Potential functions	Proposed mechanisms	Clinical relevance	Ref.
GAS5	Up-regulated in mevastatin-treated diabetic wounds	Facilitates keratinocyte migration	Interacts with c-Myc mRNA and down-regulates the c-Myc protein	Therapeutic target; Monitor for therapeutic effects	(37)
H19	Up-regulated during keratinocyte differentiation	Accelerates keratinocyte differentiation	Sponges miR-130b-3p to up-regulate DSG1	Unknown	(45)
PRANCR	Up-regulated in primary epidermal keratinocytes	Up-regulates keratinocyte proliferation and differentiation	Stimulates the binding of E2F/ FOXM1 complex and the CHR site of the target genes (CDK1, CCNB1, CCNB2 and CDC25C) through down-regulating CDKN1A	Unknown	(42)
uc.291	Up-regulated in differentiating human epithelial keratinocytes	Promotes keratinocyte differentiation	Binds to ACTL6A, promoting the binding of BRM/BRG1 and the promoters of LOR, FLG and LCEB1	Unknown	(51)

Abbreviations: CHR, cycle genes homology region; DSG1, desmoglein1; FLG, Filaggrin; LCEB1, late cornified envelope 1B; LOR, loricrin.

has been found that ACTL6 blocks the differentiation-promoting effect of BAF, mainly by specifically affecting the binding and activation of BRM/BRG1 to the promoters of gene LOR, FLG and LCE1B (late cornified envelope 1B).[48-50] When HEKn cells were during proliferation, the direct bindings of LOR, FLG and LCE1B promoters to ACTL6A were enhanced, while the bindings to BRM/BRG1 were decreased, and the opposite results were shown during differentiation. In conclusion, uc.291 may directly bind to ACTL6A and reduce its binding to LOR, FLG and LCEB1, promoting the binding and activation of BRM/BRG1 with the mentioned target gene promoters, thus accelerating keratinocyte differentiation.[51]

Overexpression of c-Myc protein was observed in the wound margin of patients with diabetic ulcers, inhibiting cell migration and wound healing.[52,53] Hu et al[54] found that GAS5 combines with the mRNA of c-Myc, thus reducing the protein expression of c-Myc in lymphoma cell line (JVM2). Consistently, the protein expression of c-Myc was decreased in HaCaT of GAS5 overexpression. It was confirmed that mevastatin may up-regulate GAS5 to reduce the mRNA and protein expression of c-Myc, thus promoting epithelialisation and diabetic wound healing.[37]

Altogether, concerning the mechanistic functions of PRANCR, H19 and uc.291 in keratinocyte metabolism in vitro, further studies should be conducted to testify their diagnostic or prognostic roles in reepithelialisation of diabetic wounds. Surprisingly, mevastatin accelerates skin wound healing in diabetes by up-regulating GAS5, implying GAS5 may serve as a target for treatment or monitor for therapeutic effects of drugs (Table 2, Figure 3).

5 | LNCRNAs AND MATURATION PHASE

Growing evidence indicates that both miRNAs[55-57] and lncRNAs[58,59] are involved in diabetic complications, and multiple angiogenic miRNA-lncRNA pairs relate to wound healing in maturation phase.[60-62] The maturation of wound healing includes angiogenesis, proliferation and migration of fibroblasts, as well as collagen deposition. LncRNA GAS5, IGF2AS, MALAT1, ANRIL, H19, MIAT and lncEGFL7OS were related to angiogenesis, while H19 and MALAT1 were also involved in proliferation and differentiation of fibroblasts, and TETILA, H19 and lnc-URIDS could possibly affect collagen deposition.

5.1 | The expression patterns of lncRNAs in diabetic patients

Mevastatin treatment not only increased the expression of GAS5 and wound healing in diabetic patients, but also accelerated angiogenesis.[37]

The expression of lncRNA-ANRIL (antisense RNA to INK4 locus) in diabetic peripheral blood was decreased, and its knockdown deteriorated lymphatic angiogenesis

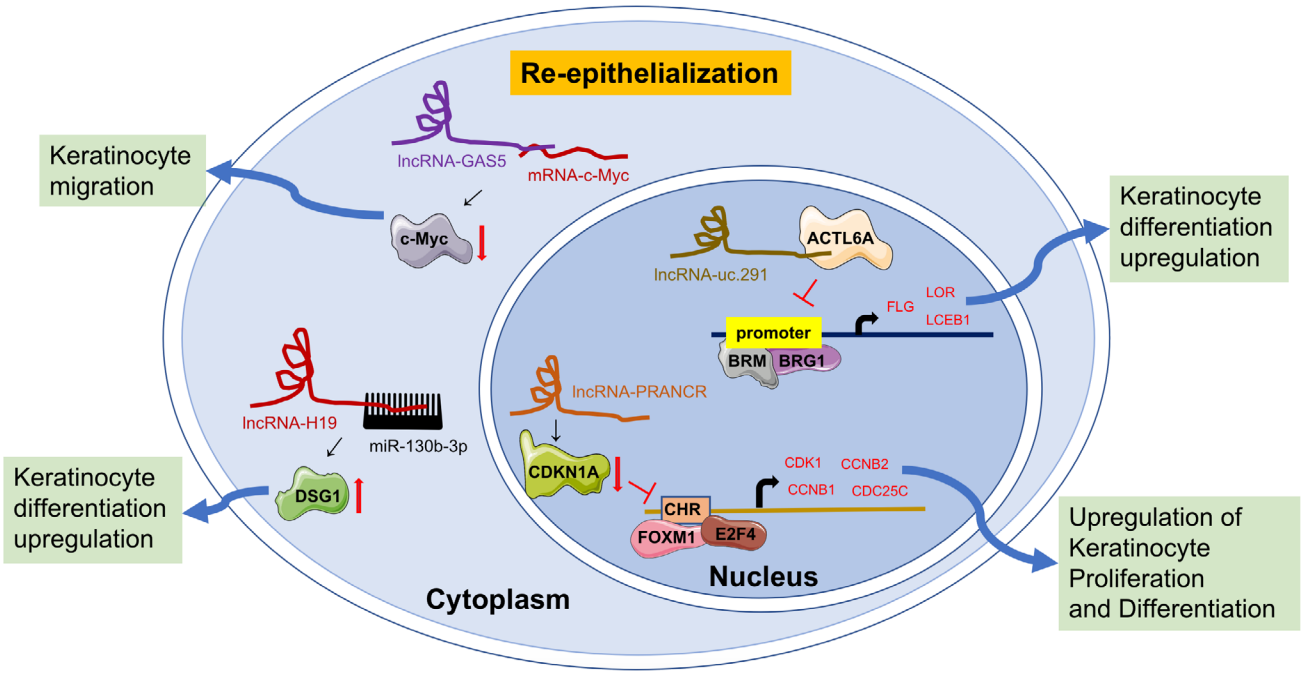

FIGURE 3 Functions of lncRNAs in reepithelialisation of skin wound healing in diabetes. LncRNA-GAS5 binds with c-Myc mRNA and down-regulates the c-Myc protein, facilitating keratinocyte migration. LncRNA-H19 sponges miR-130b-3p to promote the expression of desmoglein1 (DSG1), accelerating keratinocyte differentiation. LncRNA-PRANCR optimises keratinocyte proliferation and differentiation by stimulating the binding between the complex of E2F and FOXM1 and the cell cycle genes homology region (CHR) site of the target genes, namely CDK1, CCNB1, CCNB2 and CDC25C, through down-regulating CDKN1A, which can bind to CHR sequence to inhibit cell homology. LncRNA-uc.291 accelerates keratinocyte differentiation through promoting the binding of BRM/BRG1 and the promoters of genes LOR, FLG, LCEB1 by directly binding ACTL6A to suppress its transcription-inhibited binding with the above promoters

marked by decreased prospero homeobox 1 (Prox1) protein.[63-65] Moreover, Prox1 can be inhibited by miR-181a, and the expression of miR-181a in serum of patients with type 2 DM is up-regulated.[66,67] It was hypothesised that ANRIL stabilises Prox1 by sponging miR-181a.[68]

The expression of H19 in umbilical cord blood of gestational DM is decreased, while the negative feedback of H19/let-7 leads to abnormal PI3K/AKT pathway and vascularization, implicating the relations between H19 and angiogenesis of DM.[69-71] Besides, higher level of H19 is detected in the ulcerative edge tissues of DFU patients than in normal tissues, which might be a key factor in wound healing.[72]

Lnc-RNA MRAK052872 (named lnc-URIDS) is dramatically increased not only in the diabetic skin, but also in the serum of type 2 DM patients with DFU, indicating its participation in wound healing of diabetes.[73]

5.2 | Regulation of lncRNAs in diabetic animal models

Surprisingly, mesenchymal stem cell (MSC) of MALAT1 overexpression treatment accelerated the wound healing in streptozocin (STZ)-induced diabetic mice.[74]

Silencing of H19 with virus injection into the edge of wound impeded the wound healing of DFU mice while overexpressing H19 promoted wound healing.[72] In the diabetic rats treated with extracellular vesicle-mimetic nanovesicles (EMNVs) carrying overexpressed H19, wound healing and angiogenesis were accelerated.[75] Furthermore, the MSC-derived exosomes containing H19 significantly expedited the wound healing by injection into the wound tissues of diabetic mice as evidenced by thicker granulation tissues.[76] Additionally, H19 may stimulate wound healing by positively regulating hypoxia induced factor-1α (HIF-1α), which is beneficial to diabetic wound healing.[77] Overexpressed H19 by lentivirus wound margin injection resulted in up-regulated protein expression of HIF-1α, promoted angiogenesis, fibroblast proliferation and collagen deposition in diabetic rats.[78] Similarly, MALAT1 could also promote angiogenesis and fibroblast viability through HIF-1α. Accelerated wound healing, increased levels of MALAT1 and HIF-1α (mRNA and protein) were observed in diabetic mice treated with modified autologous blood.[79]

In the skin tissue of STZ-induced diabetic rats, lnc-URIDS is significantly increased and mainly expressed in the dermal fibroblasts. Rats treated with

adenovirus-expressing lnc-URIDS exhibited a wider wound, sparser deposition of collagen and decreased ratio of collagen I/III compared with rats treated with control adenovirus at day 10 post-wounding, while suppressing the expression of lnc-URIDS with RNA interference (RNAi) showed the opposite. These results indicate that inhibition of lnc-URIDS may significantly ameliorate cutaneous wound healing of diabetes.[73]

5.3 | The potential molecular mechanisms of lncRNAs

In epithelial keratinocytes, the mRNA expressions of GAS5, VEGFA and heparin binding EGF like growth factor (HBEGF) were increased by mevastatin, indicating that mevastatin promoted angiogenesis and wound healing by up-regulating GAS5.[37]

Researchers observed reduced levels of insulin-like growth factors (IGF)-1 and IGF2 in the blood of diabetes, leading to angiopathy.[80-82] In myocardial microvascular endothelial cells (mMVE) of diabetic rats, the mRNA expression of IGF2 was decreased, while the level of lncRNA-IGF2AS (antisense RNA of IGF2) increased. Surprisingly, down-regulation of IGF2AS not only enhanced the cell proliferation and migration, but also increased the mRNA and protein expression of IGF2, VEGF and IGF1 in mMVE. However, when the expression of IGF2 was knockdown, the mRNA and protein expression of VEGF, cell proliferation and migration decreased despite of IGF2AS knockdown. In brief, IGF2AS may attenuate angiogenesis by negatively regulating IGF2.[83]

Bioinformatics analysis predicted a potential binding site between miR-152-3p and MALAT1 in MSC, and it was confirmed that MALAT1 acted as the ceRNA of miR-152-3p. In MSC, overexpression of MALAT1 led to decreased miR-152-3p and increased level of VEGF protein. In human endothelial cells treated with MSC overexpressing MALAT1, the tube formation was increased, indicating the therapeutic role of MALAT1 in vascular defects.[74]

According to the down-regulation of ANRIL, Prox1 protein and the up-regulation of miR-181a in diabetic patients, He et al[68] shed light on the molecular mechanisms in human lymphatic endothelial cells and found that ANRIL may ameliorate the stable expression of Prox1 and lymphatic angiogenesis through sponging to miR-181a.

The levels of H19, activation of AKT, cell migration and vascularization were decreased in HG-culture human skin microvascular endothelial cells (HMEC-1), and overexpressing H19 increased cell migration and angiogenesis.[75] Additionally, the expression of H19 in skin fibroblasts of STZ mice was lower than that of normal fibroblasts. In the Db mouse fibroblasts, H19 overexpression ameliorated the proliferation and migration of fibroblasts, up-regulated the protein expression of HIF-1α and the level of H3K4me3 (trimethylation of lysine 4 on histone H3 protein subunit) on HIF-1α promoter. The binding of H19 to the promoter of HIF-1α on region 1402 to 1414 was predicted and confirmed. Another study indicated that H19 binds to enhancer of zeste homologue 2 (EZH2) in diabetic fibroblasts and the expressions of EZH2 and H3K4me3 increased with the increase of H19.[84] Furthermore, the direct binding of EZH2 and H3K4 resulted in the increase level of H3K4me3 and HIF-1α in fibroblasts. Comprehensively, H19 may recruit EZH2 to combine with H3K4, increasing the activation of H3K4me3, which binds to the promoter region of HIF-1α, thus facilitating the transcription of HIF-1α and wound healing. Similarly, H19 may also elevate connective tissue growth factor (CTGF) expression by recruiting serum response factor (SRF) to the promoter region of CTGF, thus accelerating cell proliferation, ECM remodelling and wound healing while repressing cell apoptosis.[85]

Li et al[72] showed that the proliferation and migration of fibroblast obtained from DFU patients were enhanced by transfecting with overexpressed-H19. It was predicted and confirmed that H19 competitively binds to miR-29b, a miRNA that may repress fibroblast proliferation and migration by inhibiting FBN1 (fibrillin 1). H19 elevates the expression of FBN1 through sponging to miR-29b, which enhances the proliferation, migration, and inhibits apoptosis of fibroblasts, thus facilitating the wound healing of DFU.

A detailed study confirmed that miR-152-3p targetedly inhibits PTEN (phosphatase and tensin homologue deleted on chromosome 10), while H19 could bind with miR-152-3p. Overexpression of H19 led to decreased miR-152-3p, apoptosis, activation of PI3K/AKT pathway, and increased PTEN, cell proliferation and migration in fibroblasts. Moreover, injecting MSC-derived exosomes containing H19 into the fibroblasts of diabetic mice expedited wound healing by accelerating proliferation, migration and inhibiting apoptosis. These results suggested that MSCs-derived exosomes carrying H19 stimulate diabetic wound healing by promoting fibroblast proliferation and migration through reducing the miR-152-3p-mediated inhibition on PTEN.[78]

In diabetic mouse skin fibroblasts and HG-cultured fibroblasts, overexpression of MALAT1 resulted in up-regulation of HIF-1α, FAP, COL1, COL3 protein and cell activity. Notably, the fibroblast activity and the acceleration of wound healing promoted by overexpression of MALAT1 could be inhibited by the knockdown of HIF-1α. In summary, MALAT1 may stimulate the fibroblast

activity through HIF-1α pathway, thus optimising diabetic wound healing.[79]

In diabetic skin, the level of MMP-9 protein increased and the collagen formation decreased.[86,87] Acknowledging, injecting the AGEs is an essential way to simulate the diabetes condition in vitro and it induces demethylation in protein ten-eleven translocation 2 (TET2) to bind with the promoter of MMP-9, impeding diabetic wound healing.[88-90] In HaCaT treated with AGEs, lncRNA-TETILA (TET2-interacting long non-coding RNA) was up-regulated most significantly, while the DNA methylation of MMP-9 decreased, resulting in up-regulation of mRNA and protein expressions of MMP-9. Knockdown of TETILA tended to promote cell migration with decreased expression of TET2 and MMP-9. It was suggested that TETILA may induce MMP-9 demethylation by enhancing the expression of TET2. Moreover, down-regulation of TET2 by TETILA knockdown was rescued by ubiquitin enzyme inhibitor, suggesting TETILA may inhibit the ubiquitin degradation of TET2 protein. Notably, the combination of TET and thymine-DNA glycosylase (TDG) leads to demethylation of DNA, and the demethylation of MMP-9 promoter induced by TETILA was reduced by TET2 or TDG knockdown.[91,92] The combination of TETILA and TDG was confirmed by RIP (RNA Binding Protein Immunoprecipitation) detection. In summary, up-regulation of TETILA in diabetes may stimulate the stable expression of TET2 protein and recruit TET2/TDG to the promoter of MMP-9, thus promoting the expression of MMP-9 protein, decreasing the formation of collagen, and attenuating wound healing.[93]

Rat dermal fibroblasts were treated with advanced glycation end products (AGEs) to establish an in vitro diabetic wound healing model, in which the expression levels of lnc-URIDS were significantly increased while the cell migration was decreased. On the contrary, knocking down the expression of lnc-URIDS in fibroblasts with RNAi, the cell migration was significantly increased, indicating that lnc-URIDS knockdown ameliorated phenotypes associated with diabetic dermal fibroblasts in vitro. Moreover, RNA pull down assay was conducted to identify the lnc-URIDS binding proteins and Plod 1 (Procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1) was screened followed by MS, which was verified by western blot analysis. Overexpression of lnc-URIDS exhibited no effects on the mRNA level of Plod 1, but decreased the protein expression of Plod 1. Further experiment showed that lnc-URIDS interacts with Plod 1 to reduce its protein stability, thus impeding diabetic wound healing.[73]

Since endothelial cells (ECs) are recognised as the primary cellular targets during diabetes-induced vascular damage,[94] HUVEC cells and HMECs cultured in HG were adopted, and up-regulated lncRNA MIAT (myocardial infarction associated transcript) was observed. It was hinted that MIAT may shed light on the angiogenesis of diabetic wound healing.[95]

To identify lncRNAs specific in ECs, a microarray was performed and lncEGFL7OS was screened out because of its EC restriction and potential relevance to EC function. Silence/overexpression of lncEGFL7OS in HUVEC cells inhibited/promoted the proliferation, migration and formation of vascular tubules, suggesting that lncEGFL7OS is required for proper angiogenesis in vitro. Because lncRNAs could exert regulatory function in cis on the neighbouring genes in the nucleus and lncEGFL7OS is located in the opposite strand neighbouring EGFL7/miR-126,[96] we surmised that lncEGFL7OS regulates angiogenesis by controlling EGFL7/miR-126 expression. Consistently, the expressions of EGFL7 (mRNA and protein) and miR-126 were dramatically decreased upon lncEGFL7O knockdown in HUVECs. Of note, the combination of miR-126 and EGFL7 could rescue the anti-angiogenic effect of lncEGFL7OS silencing, indicating that lncEGFL7OS promoted angiogenesis by maintaining maximal expression of EGLF7/miR-126. Furthermore, lncEGFL7OS was predicted to interact with MAX transcription factor and it was confirmed by experiments that MAX bound to the bidirectional lncEGFL7OS/EGFL7/miR126 promoter/enhancer. Overexpression of lncEGFL7OS significantly increased the above binding activity. These results manifested that lncEGFL7OS positively regulated angiogenesis by interacting with MAX at EGFL7/miR126 locus, whereas the mechanism in diabetic condition awaits further investigation.[62]

To conclude, the differential expressions of GAS5, ANRIL, H19 and lnc-URIDS in diabetic patients show their potential roles as biomarkers for vascular defects of DM. Additionally, studies in diabetic animal model demonstrated that H19, MALAT1 and lnc-URIDS might be potential therapeutic targets of diabetic ulcers. In terms of IGF2AS, TETILA MIAT and lncEGFL7OS, although the molecular mechanisms of which except MIAT have been partially elucidated in vitro, more in vivo studies are necessary to support the hypothesis that they could be promising predictive biomarkers and potential therapeutic targets in diabetic wounds (Table 3, Figure 4).

6 | LNCRNAs INVOLVEMENT IN NEUROPATHY

Diabetic neuropathy is secondary to the increase of blood glucose level and mainly involves the peripheral nerves of the lower extremities. Diabetic peripheral neuropathy (DPN) is the most shared diabetic neuropathy, affecting over 50% of diabetic patients.[97] The main manifestations were obstruction of sensory and motor nerve conduction, decreased expression of neuropeptides and imbalance of pro-inflammatory and anti-inflammatory cytokines.[98]

TABLE 3 lncRNAs and maturation phase

lncRNA	Expressions	Potential functions	Proposed mechanisms	Clinical relevance	Ref.
GAS5	Up-regulated in mevastatin-treated diabetic wounds	Improves angiogenesis	Up-regulates the mRNA levels of VEGFA and HBEGF	Therapeutic target; Monitor for therapeutic effects	(37)
H19	Down-regulated in blood of gestational DM, skin fibroblasts of diabetic mice and HG-cultured HMEC-1	Up-regulates angiogenesis, collagen deposition and fibroblast proliferation	Sponges miR-152-3p/miR-29b to up-regulate PTEN/FBN1; Recruits SRF to the promoter region of CTGF; Recruits EZH2 to bind with H3K4, increasing the expression of histone H3K4me4 and promoting the transcription of HIF-1α	Biomarker; Therapeutic target	(72,78,84)
ANRIL	Down-regulated in diabetic peripheral blood	Promotes lymphatic angiogenesis	Sponges miR-181a and stabilises the expression of Prox1	Biomarker	(63,68)
MALAT1	Up-regulated in peripheral vein of diabetic mice treated with modified autologous blood	Optimises angiogenesis and fibroblasts viability	Sponges miR-152-3p; Activates HIF-1α signalling pathway	Therapeutic target	(74,79)
IGF2AS	Up-regulated in mMVE of diabetic rats	Impairs angiogenesis	Down-regulates IGF2 and VEGF	Unknown	(83)
Lnc-URIDS	Up-regulated in diabetic skin and serum of DFUs	Impairs collagen deposition	Interacts with Plod 1 to reduce its protein stability	Biomarker; Therapeutic target	(73)
TETILA	Up-regulated in AGEs treated HaCaT cells	Down-regulates collagen deposition	Interacts with TDG and attenuated the ubiquitin degradation of TET2	Unknown	(93)
MIAT	Up-regulated in HG-cultured HUVEC cells and HMECs	Regulates angiogenesis	Unknown	Unknown	(95)
lncEGFL7OS	Up-regulated dilatedCardiomyopathy patients	Promotes angiogenesis	Interacts with MAX at EGFL7/miR126 locus	Unknown	(62)

Abbreviations: EZH2, enhancer of zeste homologue 2; FBN1, fibrillin 1; H3K4me4, trimethylation of lysine 4 on histone H3 protein subunit; HBEGF, heparin binding EGF like growth factor; HG, high glucose; HIF-1α, hypoxia induced factor-1α; HMEC-1, human skin microvascular endothelial cells; IGF, insulin-like growth factors; mMVE, myocardial microvascular endothelial cells; Plod 1, procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1; Prox1, prospero homeobox 1; PTEN, phosphatase and tensin homologue deleted on chromosome 10; SRF, serum response factor; TDG, thymine-DNA glycosylase; TET2, ten-eleven translocation 2; VEGFA, vascular endothelial growth factor A.

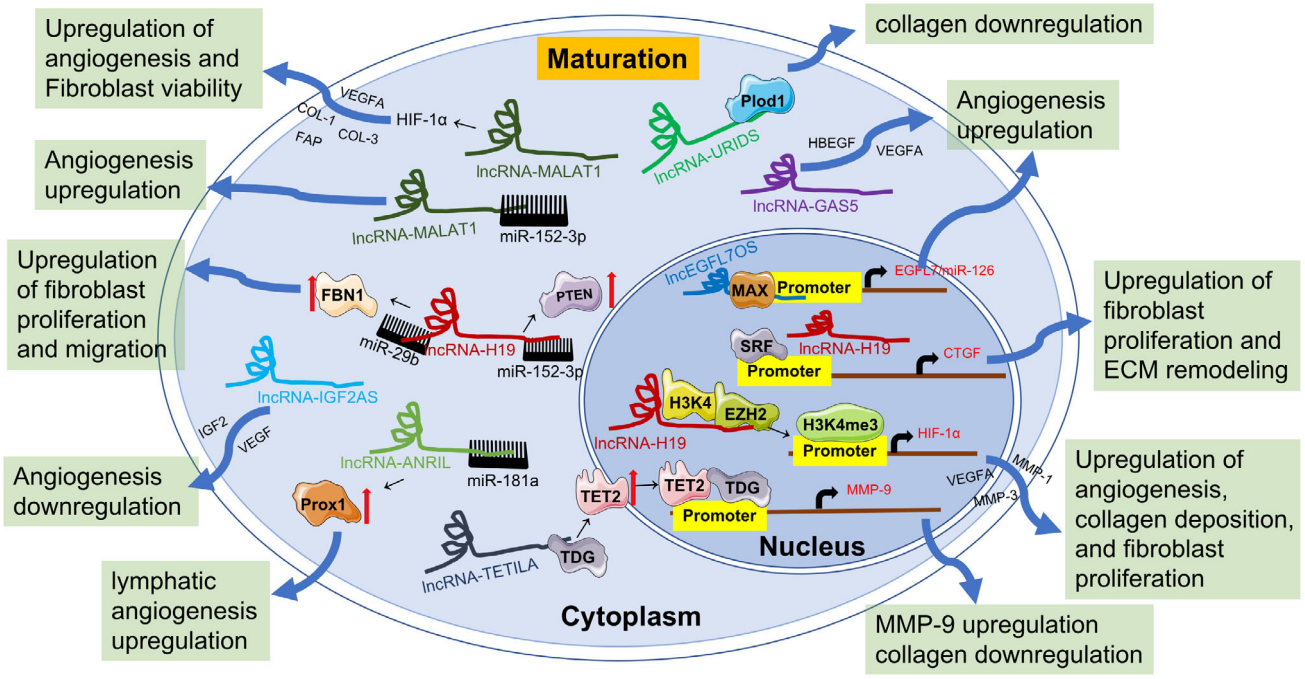

FIGURE 4 Mechanisms of lncRNAs in maturation of cutaneous healing in diabetes. LncRNA-GAS5 improves angiogenesis via up-regulation of vascular endothelial growth factor A (VEGFA) and heparin binding EGF like growth factor (HBEGF). LncRNA-H19 combines miR-152-3p or miR-29b to increase the protein expression of phosphatase and tensin homologue deleted on chromosome 10 (PTEN) or fibrillin 1 (FBN1), resulting in up-regulation of fibroblast proliferation and migration. Furthermore, lncRNA-H19 recruits enhancer of zeste homologue 2 (EZH2) to bind with H3K4, increasing the expression of histone H3K4me4, which can bind to the promoter of hypoxia induced factor-1α (HIF-1α) and promote the transcription of HIF-1α, thus promoting diabetic wound healing by up-regulating angiogenesis, collagen deposition and fibroblast proliferation. H19 may also elevate connective tissue growth factor (CTGF) expression by recruiting serum response factor (SRF) to the promoter region of CTGF, thus accelerating fibroblast proliferation, ECM remodelling and wound healing. LncRNA-ANRIL sponges miR-181a to reduce its targeted inhibition on Prospero Homeobox 1 (Prox1), thus promoting lymphatic angiogenesis. LncRNA-MALAT1 promotes diabetic wound healing not only through sponging miR-152-3p to ameliorate angiogenesis, but also facilitating fibroblast viability through HIF-1α pathway. LncRNA-IGF2AS inhibits the proliferation and migration of mMVE to impair angiogenesis by negatively regulating insulin-like growth factor 2 (IGF2) and VEGFA. Lnc-URIDS interacts with protein procollagen-lysine, 2-oxoglutarate 5-dioxygenase 1 (Plod 1) to reduce its protein stability, thus down-regulating collagen deposition. LncRNA-TETILA restrains the ubiquitin degradation of TET protein to promote its stable expression, and ten-eleven translocation 2 (TET2) further binds with thymine-DNA glycosylase (TDG) to mediate the demethylation of matrix metalloprotein 9 (MMP-9) promoter, promoting the expression of MMP-9 protein, decreasing the formation of collagen and impairing wound healing. LncEGFL7OS recruits and binds with MAX to enhance the transcription of EGFL7/miR-126 gene, therefore promoting angiogenesis

Many research studies suggested that lncRNAs may relate to it.

6.1 | lncRNAs and pain sensation

Dorsal root ganglion (DRG) is extremely sensitive to diabetes, and its response to hyperglycemia may lead to diabetic neuropathy.[99] A recent study showed that the expression of lncRNA-PVT1 (plasmacytoma variant translocation 1), the mechanical withdrawal threshold (MWT), thermal withdrawal latency (TWL) and the sensory nerve conduction velocity (SNCV) were down-regulated in the DRG of diabetic rats. Interestingly, overexpression of PVT1 up-regulated the MWT, TWL, SNCV and the levels of neurogenesis-related genes (Drd2, Notch1 and S100b), while down-regulated the expression of neurodegeneration-related genes (Uchl1, Sod1), indicating that PVT1 may ameliorate neurogenesis.[100]

6.2 | lncRNAs and neuropeptide levels

Neuropeptides are released by autonomic nerve fibres and cells in the dermis and epidermis, involved in the regulation of cytokines including IL-1, IL-6, IL-8, IL-10 and TNF-α.[101,102] However, the regulatory mechanism of neuropeptide and lncRNAs in diabetic ulcers remains elusive.

6.3 | lncRNAs and cholinergic anti-inflammatory effects

6.3.1 | The expression patterns of lncRNAs in DPN

To understand the roles of lncRNAs in DPN, Guo et al[103] adopted RNA-seq and found that lncRNA Vmuret.69.aSep08, mordey.aSep08-unspliced and mRNARGD7594602_1 were up-regulated most significantly in the DRG of DPN rats, correlated to the regulation of metabolic pathways, oxidative phosphorylation and calcium signalling pathway. In the meantime, the decrease of lncRNA Itgb8.eSep08-unspliced, veyly.aSep08-unspliced and klygu.aSep08-unspliced was the most obvious, which may be regulated with PI3K-Akt signalling pathway, peroxisome proliferator-activated receptor (PPAR) signalling pathway and MAPK signalling pathway. Recent studies have found different expressions of miR-146a-5p between type 2 diabetes and DPN.[104,105] The target gene of miR-146a-5p, involving TRAF6, IRAK1 and SMAD4, all related to inflammation. Furthermore, lncRNA XR_589933, XR_351905, XR_357013 and XR_589615 were found to be up-regulated in DPN sciatic nerve. LncRNA XR_589933, XR_351905, XR_357013 and XR_589615 may act as ceRNAs for miR-146a-5p to promote neuroinflammation of DPN.[106]

6.3.2 | The potential molecular mechanisms of lncRNAs

The expressions of lncRNA-NC021972, mRNA and protein of P2X7 receptor, p38 phosphorylation and IL-6, TNF-α protein were increased in HG-cultured PC12 cells. Knockdown of NC021972 down-regulated the expressions of mRNA and protein of P2X7 receptor. The levels of P2X7 protein and IL-6, TNF-α protein were decreased by inhibitor of MAPK pathway, suggesting that NC021972 may positively regulate P2X7 through MAPK signal pathway and lead to neuroinflammation.[107]

In summary, PVT1 and identified lncRNAs by RNA-seq may play salient roles in neuropathy of diabetes, implying their diagnostic and prognosis effects in diabetic ulcers. However, the molecular mechanisms await intensive disquisition. Although NC021972 may be critical to neuroinflammation induced by HG, validation of its function in diabetic wounds in vivo is required (Table 4, Figure 5).

7 | THE CLINICAL RELEVANCE OF LNCRNAs IN DIABETIC WOUNDS

Currently, the treatment of diabetic ulcers remains an unsolved problem. This review summarises a series of

TABLE 4 lncRNAs involvement in neuropathy

lncRNA	Expressions	Potential functions	Proposed mechanisms	Clinical relevance	Ref.
PVT1	Down-regulated in DRG of diabetic rats	Up-regulates pain sensory	Stimulates MWT, TWL and SNCV	Unknown	(100)
NC021972	Up-regulated in HG-cultured PC12 cells	Aggravates neuroinflammation	Stimulates P2X7 through MAPK pathway	Unknown	(107)
Vmuret.69.aSep08	Up-regulated in DRG of DPN rats	Unknown	Unknown	Unknown	(103)
mordey.aSep08-unspliced	Up-regulated in DPN rats' DRG	Unknown	Unknown	Unknown	(103)
mRNARGD7594602_1	Up-regulated in DRG of DPN rats	Unknown	Unknown	Unknown	(103)
Itgb8.eSep08-unspliced	Down-regulated in DPN rats' DRG	Unknown	Unknown	Unknown	(103)
veyly.aSep08-unspliced	Down-regulated in DRG from DPN rats	Unknown	Unknown	Unknown	(103)
klygu.aSep08-unspliced	Down-regulated in DRG of DPN rats	Unknown	Unknown	Unknown	(103)
XR_589933	Up-regulated in DPN sciatic nerve	Unknown	Unknown	Unknown	(106)
XR_351905	Up-regulated in sciatic nerve of DPN	Unknown	Unknown	Unknown	(106)
XR_357013	Up-regulated in DPN sciatic nerve	Unknown	Unknown	Unknown	(106)
XR_589615	Up-regulated in sciatic nerve of DPN	Unknown	Unknown	Unknown	(106)

Abbreviations: DPN, diabetic peripheral neuropathy; DRG, dorsal root ganglion; HG, high glucose; MWT, mechanical withdrawal threshold; SNCV, sensory nerve conduction velocity; TWL, thermal withdrawal latency; MAPK, mitogen-activated protein kinase.

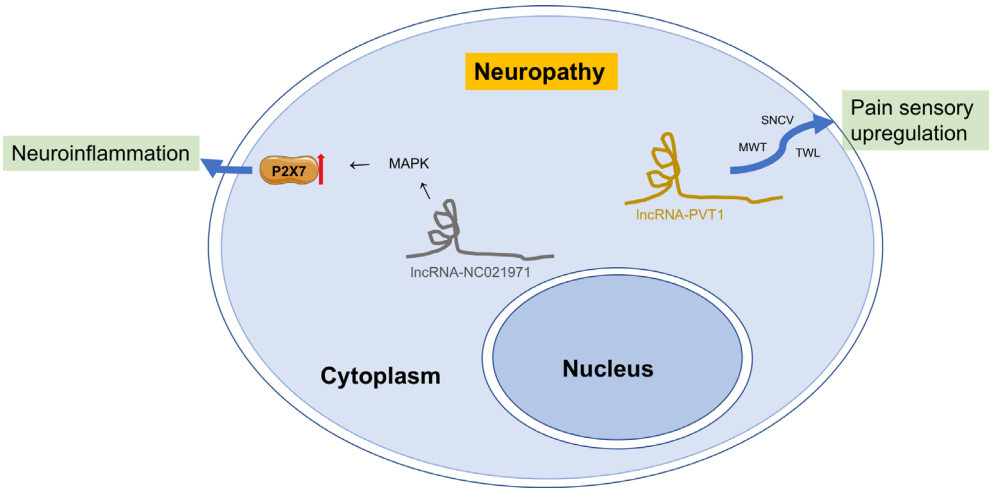

FIGURE 5 Related lncRNAs in neuropathy of diabetes associated wound healing defects. LncRNA-PVT1 promotes mechanical withdrawal threshold (MWT), thermal withdrawal latency (TWL), sensory nerve conduction velocity (SNCV), up-regulating pain sensory. LncRNA-NC021972 promotes the mRNA and protein expressions of P2X7 through mitogen-activated protein kinase (MAPK) signal pathway, leading to neuroinflammation SNCV

lncRNAs, partial of which may be utilised as diagnostic biomarkers or therapeutic targets for diabetic wounds (Figure 6).

7.1 | lncRNAs as diagnostic markers for diabetic wounds

H19 was significantly up-regulated in the blood samples of patients with DM, and its expression significantly correlated with the level of blood glucose.[29] It is suggested that H19 may potentially distinguish between diabetes and healthy controls, serving as a novel biomarker for disease activity of diabetes. Lnc-URIDS dramatic increased in the skin and serum samples of DFU patients may be a key factor leading to delayed wound healing of DM, providing evidence for diabetic ulcer diagnosis.[73] Besides, ANRIL in diabetic peripheral blood was decreased significantly and MT1P3 in blood megakaryocytes was increased.[21,63] It may contribute to diagnosis of DM by detecting the expressions of H19, ANRIL and MT1P3 in the blood samples of patients. Moreover, GAS5 was overexpressed in the skin tissues of DFU patients and mainly located in the M1 macrophages.[26] Hence, highly expressed GAS5 in M1 macrophages of DFUs skin may be biomarker for diabetic wounds in the early inflammation phase. In addition, RNA-seq identified that lncRNA-ENST00000411554 was down-regulated in skin tissues of DFU compared with non-DFU.[24] Briefly, lncRNAs isolated from body fluids or tissues may serve as sensitive diagnostic markers for diabetic wounds. Unfortunately, the clinical studies conducted to date is not convinced enough to verify their clinical value. Thus, larger patient cohorts are needed to validate the potential of these lncRNAs as clinical biomarkers.

7.2 | lncRNAs levels monitor therapeutic effects

GAS5 was up-regulated in epithelial keratinocytes after mevastatin treatment,[37] indicating changes in the GAS5 level may reflect a previously unknown effect of mevastatin therapy and may become a biomarker monitoring therapeutic effects. Besides, the diabetic mice treated with modified autologous blood showed accelerated wound healing with increased MALAT1 expression, indicating the role of MALAT1 as monitor for therapeutic effects in diabetic wounds.[79] Preclinical studies on lncRNA-based diabetic wounds therapy have made progresses and offered a new avenue for diabetic ulcer treatment, yet this needs further clinical validation for the DM patient cohorts used in previous studies are not enough and a convenient and quick technique should be developed to detect the target lncRNAs in diabetic patients.

7.3 | Development of lncRNA-targeted diabetic wound therapies

Emerging studies on lncRNA have uncovered fresh information on diabetic wound healing. However, targeting lncRNAs to treat diabetic ulcers is still challenging. Lack of related proteins means that only

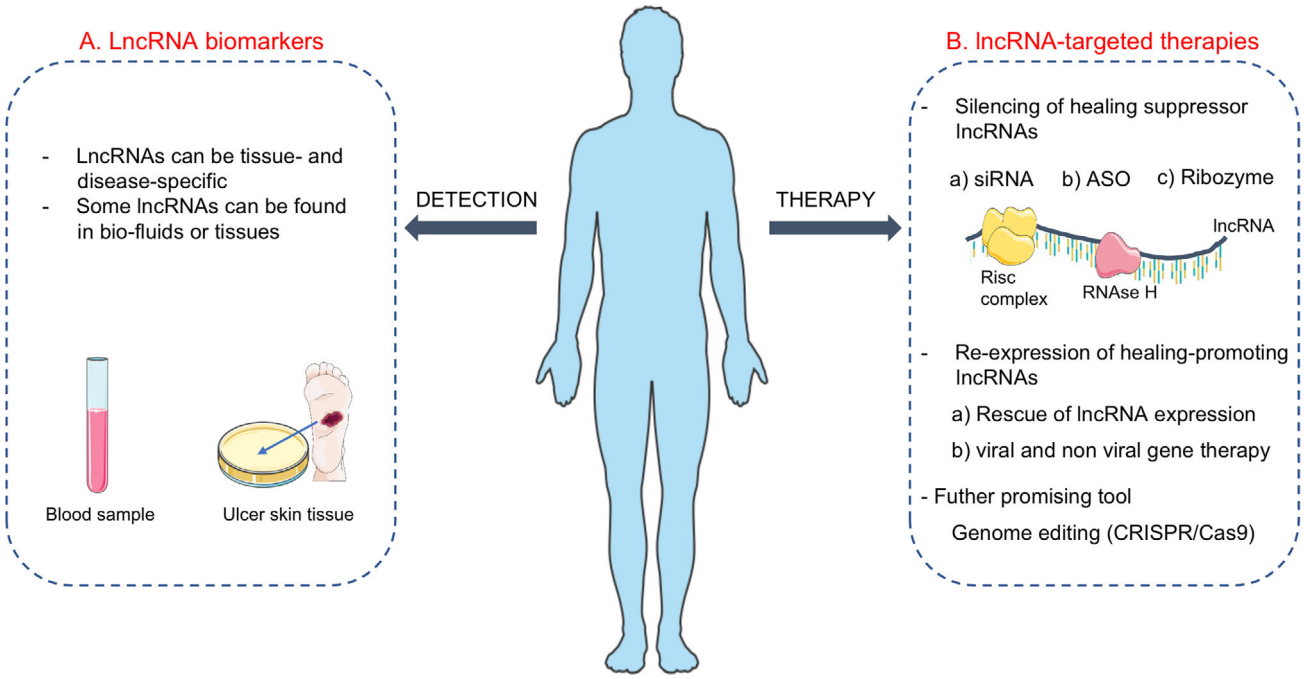

FIGURE 6 The clinical relevance of lncRNAs in diabetic wound healing. A, LncRNA biomarkers. LncRNAs are promising predicted biomarkers as their expressions are tissue- or disease-specific and some lncRNAs can be detected in the blood samples or ulcer skin tissues of diabetic patients. B, LncRNAs targeted therapies. For silencing healing-impaired lncRNAs: As double-stranded RNA oligonucleotides antisense and complementary to target lncRNA sequences, small interfering RNA (siRNA) recruits the RNA-induced silencing complex (RISC) to induce degradation of the target lncRNAs. As a single-stranded DNA oligonucleotide that is complementary to the target lncRNAs, antisense oligonucleotide (ASO) induces degradation by recruiting RNase H. Ribozymes (ribonucleic acid enzymes), a single-stranded RNA, binds lncRNAs targets and catalyses their deregulation by adopting specific conformations. For up-regulating the expressions of healing-promoted lncRNAs: Re-expression of the specific lncRNAs may be induced by common gene therapy strategies, packaging the whole transcript into viral or non-viral delivery tools. New Genome editing strategies involving CRISPR/Cas9 are developing and seem to be a powerful candidate to knock-in or knock-out lncRNAs

RNA-based tools are available for lncRNA applications and the lncRNA database is relatively imperfect.[108] Also, protecting samples from degradation is arduous. Furthermore, it is difficult to develop structure-based strategies for the poorly understood conformations of lncRNA.[109] Surprisingly, approaches for re-establishing the homeostatic levels of mRNA could be extended to lncRNAs, involving viral or non-viral delivery system, CRISPR/Cas9 gene editing strategy and RNAi technology with small interfering RNAs (siRNAs), small hairpin RNAs (shRNAs) and antisense oligonucleotides (ASOs).[110,111] For instance, injecting overexpressed H19 carried by EMNVs, lentivirus or exosomes, silencing lnc-URIDS with RNAi ameliorated diabetic wound healing in vivo.[73,75,76,84] However, these studies are preliminary and great challenges lie ahead for applying these treatment strategies to clinic, requiring efforts in oligonucleotide chemistry and development of appropriate delivery systems.

8 | CONCLUSIONS AND OUTLOOKS

Recently, progress has been made on discovering diabetes-related lncRNAs, understanding their functions in diabetic ulcers pathology and revealing the mechanisms. LncRNAs H19, GAS5 and lnc-URIDS were observed in both studies in human and animal studies, as well as cell experiments, and the salient roles of H19 and GAS5 in diagnosis or therapies of diabetic wound healing were demonstrated all in inflammation, reepithelialisation and maturation.[27,29,37,45,72,78,84] As for MALAT1, in vitro and in vivo studies have confirmed that it participates in inflammation of diabetic wound healing and may serve as a therapeutic target for accelerating maturation.[27,64,79] In terms of the relations of lncRNAs and neuropathy, studies have found that lncRNAs are associated with diabetic neuropathy by RNA-seq, but lacks further experimental verification in vivo and in vitro.

The findings illustrated here provide novel insights into the development of novel lncRNA-based strategies to diabetic wound therapy. However, it is undeniable that our understanding of gene regulation by lncRNA in diabetic wounds is still in the early stage. Based on the characteristics of lncRNAs and existing techniques, application of lncRNAs to diabetic wounds clinically represents a tremendous challenge. Hence, more in-depth research studies are necessary to achieve greater breakthroughs. We look forward that lncRNA-based diagnosis, monitoring and therapeutic approaches will be prevalent in diabetic patients and will play a major role clinically in the future.

CONFLICT OF INTEREST

No potential conflicts of interest relevant to this article were reported.

AUTHOR CONTRIBUTIONS

Shuang-Yi Yin: Designed the work. **Le Kuai** and **Jing-Si Jiang:** Drafted and wrote the manuscript. **Bin Li** and **Wei Li:** Evaluated the manuscript. All authors read, provided feedback and approved the final protocol.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study

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