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Review

Targeting DNA methylation and demethylation in diabetic foot ulcers

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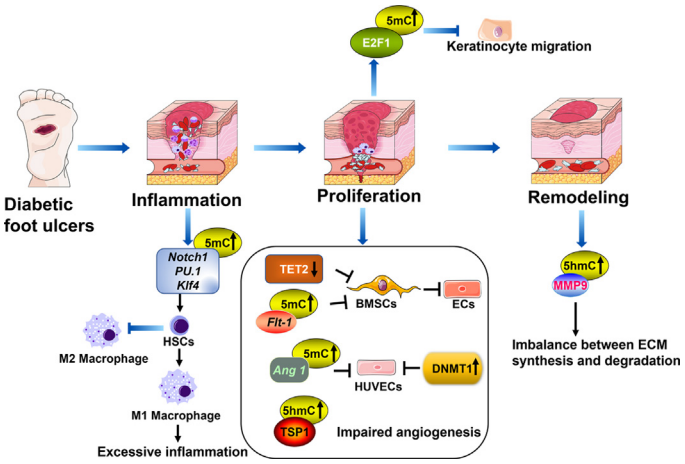
HIGHLIGHTS

- Introduced the four main phases of wound healing and their abnormality in diabetic patients.
- DNA methylation and demethylation regulate diabetic wound healing in different types of cells.
- Potential application of cellular reprogramming technology to improve diabetic wound healing.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Poor wound healing is a significant complication of diabetes, which is commonly caused by neuropathy, trauma, deformities, plantar hypertension and peripheral arterial disease. Diabetic foot ulcers (DFU) are difficult to heal, which makes patients susceptible to infections and can ultimately conduce to limb amputation or even death in severe cases. An increasing number of studies have found that epigenetic alterations are strongly associated with poor wound healing in diabetes.

Aim of review: This work provides significant insights into the development of therapeutics for improving chronic diabetic wound healing, particularly by targeting and regulating DNA methylation and demethylation in DFU.

Key scientific concepts of review: DNA methylation and demethylation play an important part in diabetic wound healing, via regulating corresponding signaling pathways in different breeds of cells, including macrophages, vascular endothelial cells and keratinocytes. In this review, we describe the four main

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phases of wound healing and their abnormality in diabetic patients. Furthermore, we provided an in-depth summary and discussion on how DNA methylation and demethylation regulate diabetic wound healing in different types of cells; and gave a brief summary on recent advances in applying cellular reprogramming techniques for improving diabetic wound healing.

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Introduction

Diabetes mellitus (DM) is a metabolic disorder having to do with chronic microvascular and macrovascular complications that poses a grave threat to human health. According to the IDF Diabetes Atlas, approximately 537 million adults were living with diabetes in 2021 and the number is estimated to amount to 783.2 million in 2045, demonstrating the increasing social burden caused by diabetes [1]. If diabetes is not well controlled and treated, diabetic patients will suffer a number of serious complications, which are important causes of death in diabetic patients. Diabetic foot ulcers (DFU) are a serious complication of diabetes, with a prevalence of 15–25 % among diabetic patients [2]. Patients with DFU face a higher economic burden and mortality than those with ordinary diabetes [3]. The treatment of DFU is very challenging and follows three crucial principles: rapid debridement, offloading and diabetic foot education. Currently, the available treatments for DFU mainly include debridement, wound unloading, dressings, glucose control, negative pressure wound therapy, skin grafting, bioengineered skin and energy therapy. Unsuccessful treatment of DFU can cause amputation or, in some severe cases, even death in diabetic patients.

Hyperglycemia is a major contributing factor to poor diabetic wound healing, but the underlying mechanism remains uncertain. A growing number of research has discovered that the adverse effects of diabetic complications persist for a long time after hyperglycemia is controlled and restored to an ideal level and that only long-term intensive glycemic control can mitigate the risk of developing diabetic complications[4,5]. This is thought to flow from the epigenetic alterations in the body's cells that are exposed to a high-glucose environment for a long time, which ultimately leads to the constant development of diabetic complications. Epigenetics appertains to heritable changes in gene expression and phenotype without altering the nucleotide sequence, mainly including histone post-transcriptional modifications, DNA methylation, non-coding RNA regulation and chromatin remodeling, all of which are reversible and can individually or synergistically affect gene expression and regulate disease states. DNA methylation is the main epigenetic regulation of gene silencing, which occurs at the cytosine 5 carbon position of cytosine-phosphate-guanine (CpG) dinucleotides, forming 5-methylcytosine (5mc) [6,7]. Although DNA methylation is the most well-studied form of epigenetic modification, the exact mechanism by which DNA methylation is involved in the pathogenesis of DFU is unclear [8–10]. This article reviews the regulation of DNA methylation and demethylation in diabetic wound healing, to provide innovative and valuable inspirations into the development of therapeutics for DFU.

Four main phases of wound healing

As illustrated in Fig. 1, the wound healing process is usually divided into four main steps: hemostasis, inflammation, proliferation and remodeling. During normal wound healing, multiple cell types act in concert to promote wound healing, including fibroblasts, endothelial cells (ECs), platelets, keratinocyte and phagocytes, and is governed by a series of growth factors.

The initial healing phase consists of hemostasis, vasoconstriction, and coagulation system activation. In diabetic patients, metabolic disturbances disrupt the physiological balance between coagulation and fibrinolysis, resulting in a platelet hypersensitivity reaction and impaired coagulation and hypofibrinolysis [11]. In the inflammatory phase, the wound site is infiltrated by inflammatory cells. Specifically, neutrophils arrive at the scene of tissue damage.

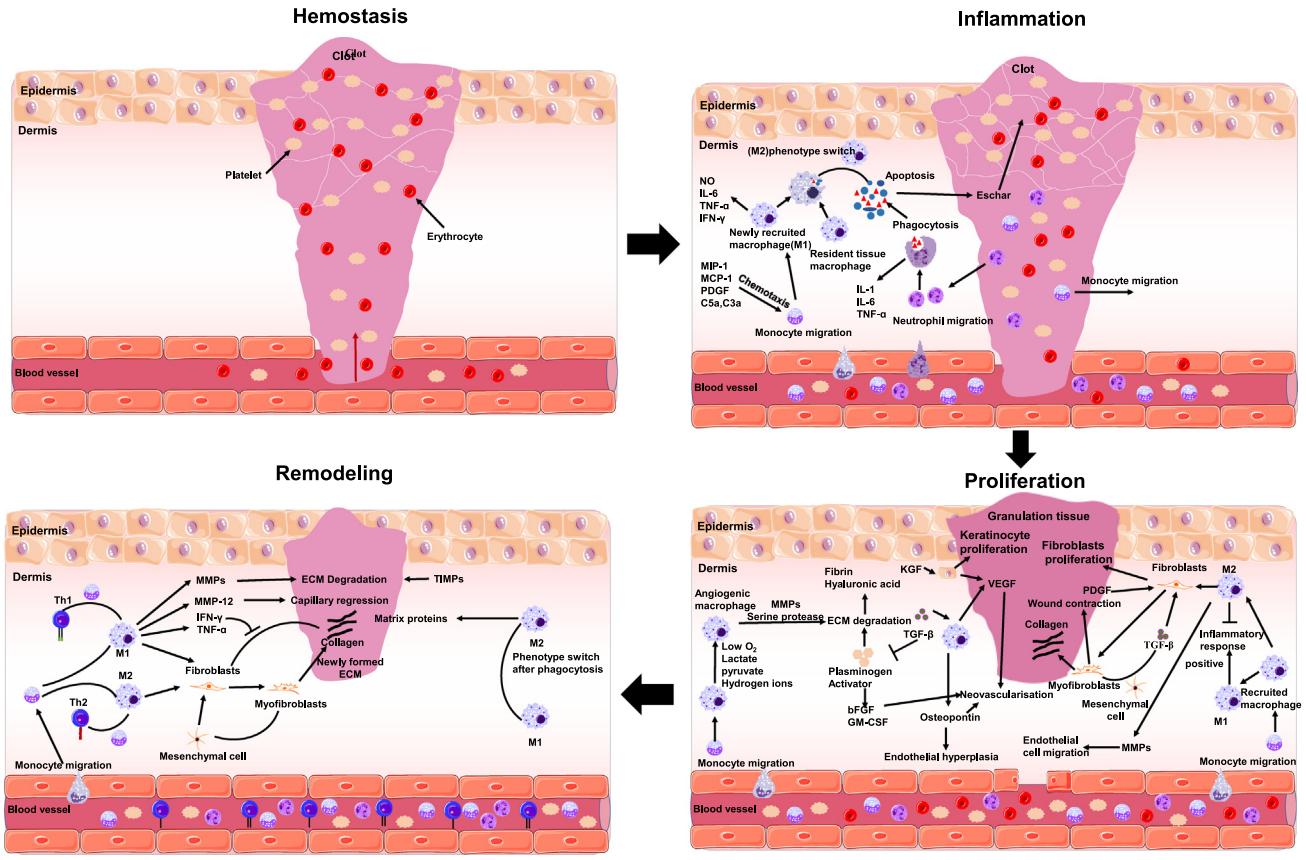

Fig. 1. Four main stages of wound healing. Wound healing begins with hemostasis, during which phase platelets bind to fibrin to form a clot and a temporary matrix. Inflammation then occurs to remove debris and prevent infection. Early inflammation is dominated by the neutrophil influx, followed by migration of monocytes into the wound and their differentiation into tissue macrophages to engulf remaining cellular debris and dead neutrophils. In the proliferative phase, angiogenesis and re-epithelialization occur, where keratinocyte migrate to bridge the wound gap, blood vessels form through neovascularization, and fibroblasts replace the initial fibrin clot with granulation tissue. In the final remodeling phase, the tissue undergoes remodeling of the ECM, repair of the barrier by myofibroblasts and contraction of the wound.

Then, monocytes enter the wound tissue, differentiate into macrophages, and operate as phagocytes to remove all stromal and cellular debris from the wound. After the inflammatory phase, wound healing enters the proliferative phase, where granulation and re-epithelialization occur, and extracellular matrix (ECM) is secreted. However, diabetic skin wounds are in a state of persistent inflammation; hence, it is not able to transmit from the inflammatory phase to the proliferative phase; thus, it will result in poor wound healing [12–14]. At the last remodeling phase of healing, the ECM expands and new wounds are remodeled to form scar tissue, where the tissue strength is restored to 80 % of the standard strength [15].

Hemostasis

Wound healing begins with hemostasis, where platelets engage with tissue collagen, and vasoconstriction play an important part in the clotting process. Specifically, the vessel wall constricts when the skin is injured in response to hemostasis. Next, platelets are rapidly recruited to the wound, and the coagulation cascade activation leads to the formation of a fibrin network. The fibrin network and platelets form a clot that binds the damaged tissue, thus stopping the wound bleeding and providing a temporary matrix for the recruitment of inflammatory cells and subsequent fibroblasts [16–18].

Inflammation

Following the hemostasis phase, wound healing undergoes inflammation. The initial stages of inflammation are dominated by neutrophils, which have three main functions: killing bacteria, secreting proteolytic enzymes to clean wounds and engulfing dead bacteria and stromal debris. Neutrophils usually undergo apoptosis after completing their task and are phagocytosed by macrophages [19]. Following the neutrophil influx, monocytes are induced by chemokines and platelet derived growth factors to migrate to the wound and to differentiate into macrophages, which then turn into the supremely critical regulatory cells during inflammatory response.

One of the crucial stages in wound healing is the removal of apoptotic cells generated by the inflammatory environment [20]. Macrophages are essential participants in the progression from the inflammatory phase to the proliferative phase in wound healing. The dynamic plasticity of macrophages allows them to mediate tissue destruction and repair functions [21]. Based on the type of stimulation, surface molecules, secreted cytokines patterns and functional characteristics, macrophages are classified into two types: classically activated macrophages (also known as M1 macrophages) and alternatively activated macrophages also named M2 macrophages [22,23]. During wound healing, M1 macrophages function to destroy pathogens, secrete pro-inflammatory factors, and participate in Th1-type responses. In contrast, M2 macrophages usually accumulate at the wound site during the repair phase of wound healing. They mainly secrete anti-inflammatory and growth factors working at the critical processes of wound healing, including angiogenesis, ECM remodeling and inflammation regression [13,24]. Fig. 1 specifically depicts various cytokines secreted by macrophages and their roles in different processes of wound healing [24,25].

In diabetic wounds, macrophages overreact to inflammation, secrete more-than-enough inflammatory factors, and are difficult to convert to the M2 phenotype with repair capability, thus bringing about a prolonged inflammatory phase [21]. In addition, due to the compromised dead-cell-clearance activity, the capability of macrophages in diabetic wounds to remove dead cells is impaired, causing an increase in the apoptotic cell load in diabetic wounds [26]. This increased apoptotic cell load produces higher levels of pro-inflammatory cytokines, which can exacerbate wound inflammation [27].

Proliferation

The proliferative phase of wound healing consists of angiogenesis and re-epithelialization. It is characterized by extensive activation of keratinocyte, fibroblasts, macrophages and ECs. During this phase, cells repopulate at the wounds and coordinate wound closure, matrix deposition, angiogenesis and ultimately the formation of new epithelial and dermal structures and vascular systems [15]. Control of these processes is under the strict regulation of biologically active factors, for instance growth factors/cytokines and the ECM environment; biologically active factors mediate the cellular adhesion/rejection interactions through degradation processes and a delicate balance between biosynthesis and cell surface receptors such as integrins. This balance is paramount, as disruption of this balance results in poor wound healing [28]. Regulation of the proliferation stage in wound healing by different types of cells and corresponding growth factors is detailed in Fig. 1 [29,30].

Angiogenesis is a principal physiological process in wound healing. It provides the re-establishment of normal blood flow, thus providing adequate oxygen and nutrient exchange and maintaining the normal metabolism of trabecular cells [31]. In healthy tissues, blood vessels remain in the basement membrane surrounding quiescent and mature vessels. During wound healing, vessels are regulated by basic fibroblast growth factor (bFGF), granulocyte–macrophage colony stimulating factor (GM-CSF) and vascular endothelial growth factor (VEGF) to promote neovascularization, while adjacent ECs begin to proliferate and migrate toward the tip cells (Fig. 1). In the normal angiogenesis process, it is important to maintain the delicate balance between promoting vascular growth and proliferation and promoting vascular maturation and quiescence. Diabetes can severely disturb this balance and cause reduced wound angiogenesis, thus disrupting normal wound healing, tissue regeneration and restoration of a healthy vascular system [32].

The wound healing process is a seriously regulated biochemical event involving the granulation of tissue formation stages and the re-epithelialization process [33]. Re-epithelialization is the reformation of epithelium in skin wounds, and keratinocytes, as the main cell group of the epidermis, take the lead in epithelialization [34]. Migration and proliferation of keratinocytes are vital steps in the wound healing process, which promotes an orderly re-epithelialization process by integrating the complex cellular processes of adhesion, migration, proliferation and differentiation through integrins and their interactions with ligands in the temporary matrix and ECM [35]. Particularly, keratinocytes not merely proliferate and migrate to cover wounds, but as well release some biomolecules, such as growth factors, chemokines and inflammatory cytokines, that regulate biologic wound healing [36,37]. The function of keratinocytes in wound healing is shown in Fig. 1. Notably, in diabetic wound healing, the proliferation and migration of keratinocytes are significantly reduced, due to the pathological state of hyperglycemia [38].

Fibroblasts are mesenchymal cells present in most tissues, whose role during the proliferative phase is to promote granulation tissue formation and replace the temporary stroma [39]. Throughout the wound healing process, fibroblasts are activated in response to tissue injury after wound injury, where fibroblasts of various origins are recruited to the wound and proliferate to fill the wound gap, thus providing new ECM and subsequently closing the wound [40]. In addition, some fibroblasts are stimulated by transforming growth factor-β (TGF-β) to differentiate into myofibroblasts, which are the main source of ECM-degrading enzymes. Myofibroblasts play a crucial role in maintaining the dynamic balance of ECM, as it promotes wound closure and tissue regeneration during wound healing [41,42]. In addition to building granulation tissue and remodeling the ECM, fibroblasts take a part in wound healing by acting as immunomodulators of wound healing [43]. In diabetic wounds, the high-glucose environment affects fibroblast differentiation, alters fibroblast apoptosis, and enhances hypoxic injury, contributing to an impaired microenvironment for myofibroblast formation, ECM regulatory disorders, and diminished wound contraction [44]. Unlike normal fibroblasts, fibroblasts in diabetic wounds exhibit premature senescence, which leads to increased levels of pro-inflammatory cytokines and type III collagen coupled with decreased levels of type I collagen and fibronectin, which further contribute to a nonhealing state [45].

Remodeling

In the final remodeling phase of wound healing, the proliferatively deposited ECM is remodeled and type I collagen replaces type III collagen, as characterized by wound contraction and scar maturation [46]. Remodeling aims to restore the normal structure of the dermis and is achieved through a delicate balance of collagen synthesis, bundling and degradation, where the matrix metalloproteinases (MMPs) and tissue inhibitor of matrix metalloproteinases (TIMPs) play a crucial role [47]. Detailed regulation of the remodeling process in wound healing by various types of cells and growth factors is summarized in Fig. 1 [48].

In diabetic wounds, the remodeling phase is prolonged owing to persistent inflammation. As confirmed by single-cell transcriptomic analysis of skin samples from non-DM patients, DM patients without DFU, and DM patients with DFU (Healers and Non-healers), coordinated actions in wound healing in DFU patients require many types of cells, including macrophages, fibroblasts, ECs and keratinocyte [49,50]. These studies also found more M1 macrophages in diabetic healing patients and more M2 macrophages in non-healing diabetic patients. This further demonstrates that delayed healing in diabetic wounds is associated with a dysregulation of the balance between M1/M2 macrophage polarization status, rather than being determined by the pro-inflammatory phenotype M1 or the anti-inflammatory phenotype M2 alone. In conclusion, poor diabetic wound healing is induced by a synergistic effect of multiple factors, and the exact mechanism is not yet clear.

DNA methylation and DNA demethylation

DNA methylation is the most abundant epigenetic modification. In mammals, it occurs almost exclusively in CpG dinucleotides, catalyzed by enzymes of the DNA methyltransferase (DNMT) family that incorporate a methyl group to the 5-C of cytosine to produce 5-methylcytosine (5mc) [51]. The main methyltransferases that regulate DNA methylation in mammals are DNMT1, DNMT3A and DNMT3B. DNMT1 was the first purified and cloned DNA methyltransferase that favors the hemimethylated CpG site and has a role in maintaining methylation in organisms. As opposed to, DNMT3A and DNMT3B are methyltransferases active on unmethylated DNA and are expressed at lower levels than DNMT1 in adult tissues. These two methyltransferases are primarily

responsible for establishing methylation patterns during early development of methylation and are involved in the maintenance of methylation sites missed by DNMT1 [52–55].

In mammals, DNA methylation can undergo both passive and active demethylation. In the absence of DNA methylation maintenance, DNA methylation is not maintained through continuous DNA replication, a phenomenon known as passive demethylation of DNA methylation. Notably, the product of DNA methylation, 5mc, can be oxidized to 5-hydroxymethylcytosine (5hmc) mediated by the ten-eleven translocation (TET) protein family, which can be further oxidized to 5-formylcytosine (5fc) and 5-carboxycytosine (5cac) catalyzed by TET proteins [56,57]. In base excision repair (BER), 5fc and 5cac can be excited and be actively reversed by thymine DNA glycosylase (TDG) to unmodified cytosine (C), a process known as active DNA demethylation [58]. TET proteases can be classified into three types: TET1, TET2 and TET3, which have a common catalytic activity but are expressed at different levels at different stages of organism development [59]. Specifically, TET1 is highly expressed in embryonic stem cells (ESCs) and primordial germ cells (PGCs), and TET2 is widely expressed in various adult tissue cells, while TET3 is the only TET enzyme present in oocytes and zygote [60,61]. Detailed schematic diagrams of DNA methylation and demethylation processes of cytosine, as well as the corresponding catalytic enzymes are illustrated in Fig. 2.

In mammals, DNA methylation and demethylation, which can co-occur in the same genomic region, are involved in various processes of tissue growth and development [62,63]. DNA methylation is often considered to be a marker of gene silencing, whereas DNA demethylation is an indicator of gene activation. DNA methylation and demethylation dynamically change within organisms, maintaining a balance in the DNA methylation status of the organism. In organisms, a normal DNA methylation status is of great importance for healthy growth and development, whereas abnormal DNA methylation is usually bound up with the development of diseases [64]. Therefore, uncovering the dynamic changes in DNA methylation and demethylation is of great importance in understanding the development of diseases.

Regulation of DNA methylation and demethylation in diabetic wounds

Due to prolonged metabolic disorders, epigenetic alterations occur in diabetic patients [65]. There are considerable variations in the status of DNA methylation and demethylation between diabetic patients and normal subjects. As DNA methylation and demethylation status can vary remarkably with one another different cell types, it is challenging to study the relationship between diabetes and DNA methylation abnormalities [66,67]. However, DNA methylation status has been demonstrated to be inextricably linked with the presence and expression of diabetes risk genes; allele-associated differential DNA methylation at CpG sites was observed in all of the 45 type 1 diabetes susceptibility genes [68,69]. Fig. 3 shows an in-depth overview of how DNA methylation and demethylation regulate diabetic wound healing, by involving in many cellular processes such as inflammation, proliferation and migration of keratinocyte, angiogenesis and MMPs secretion et al. [70–73]. Notably, both DNA methylation and demethylation are abnormal in diabetic wound healing under high-glucose conditions, resulting in the emergence of metabolic memory [74]. Table 1 summarizes the aberrant DNA methylation and demethylation status in different diabetic wound environment and their impacts on diabetic wound healing and the genes involved.

DNA methylation and macrophage polarization in diabetic wound healing

Chronic inflammation in diabetic wounds is a crucial contributor to poor diabetic wound healing. In vivo analysis of inflamma-

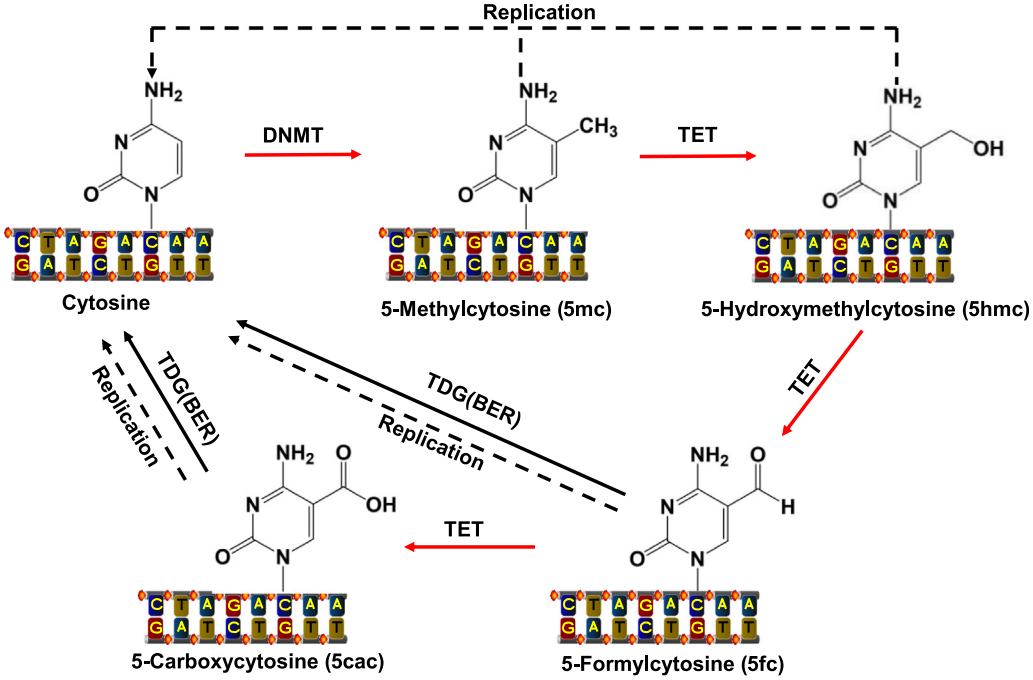

Fig. 2. DNA methylation and demethylation processes. The 5th carbon position of the cytosine can be methylated by DNA methyltransferase (DNMT) to produce 5-methylcytosine (5mc). The methyl group of 5mc can be oxidized by the 10–11 translocation (TET) family of enzymes to produce 5-hydroxymethylcytosine (5hmc). 5hmc can be further oxidized by TET to 5-formylcytosine (5fc) and 5-carboxycytosine (5cac). 5mc, 5hmc, 5fc and 5cac are reduced during DNA replication due to their inability to be maintained, a demethylation process called passive demethylation. In addition, 5fC and 5caC can be actively demethylated by thymidine DNA glycosylase (TDG) in combination with base excision repair (BER), also known as active demethylation.

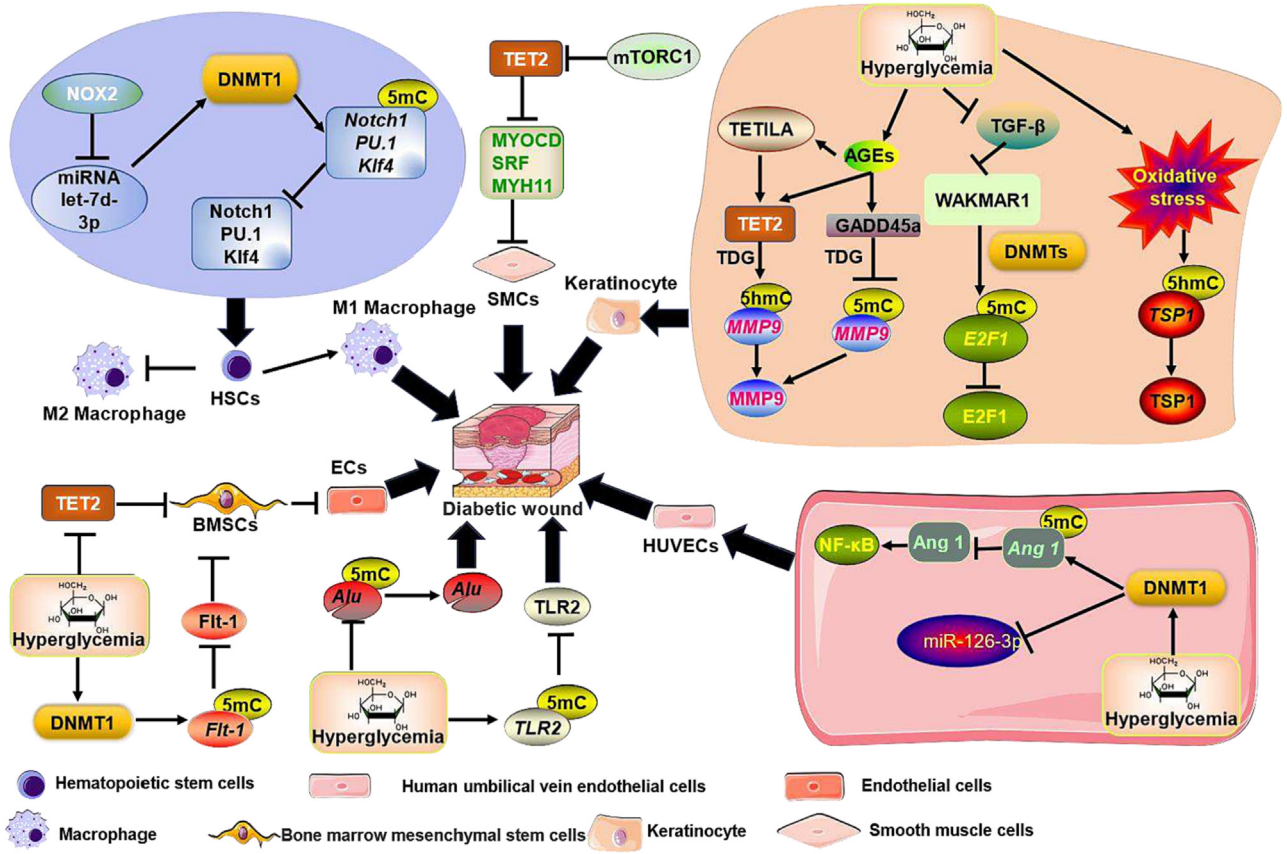

Fig. 3. Regulation of DNA methylation and demethylation in diabetic wound healing, by mediating cellular processes in different types of cells. In diabetic ischemic muscle, DNA methylation is upregulated in the promoter of M1 gene and is downregulated in the promoter of M2 gene, causing delayed wound healing. In bone marrow-derived hematopoietic stem cells (HSCs) of diabetic mice, oxidative stress induced by *NOX-2* causes DNA hypermethylation in *Notch1*, *PU.1* and kruppel-like factor (*Klf4*) genes, resulting in upregulated M1 macrophages and delayed wound healing. Methylation of the promoter of TLR2 gene downregulates the protein level of TLR2, which causes poor diabetic wound healing. Alu (B1 in rodents) hypomethylation, commonly found in diabetes mellitus patients, increases DNA damage and delays the healing process. In bone marrow mesenchymal stem cells (BMSCs), high-glucose condition was found to destabilize TET2, which in turn impairs wound healing. TET2, whose expression is negatively regulated by mTORC1, can modify and regulate the activity of promoters of key genes in smooth muscle cells (SMCs), thus enhancing SMC differentiation and improving vascular repair in wound healing. In human umbilical vein endothelial cells (HUVECs), due to elevated DNMT1, Ang-1 gene is hypermethylated and its protein level is downregulated, causing sustained activation of nuclear factor-κB (NF-κB) and subsequent endothelial dysfunction. However, overexpression of DNMT1 downregulated the expression levels of miR-126-3p and Flt1, thus impairing angiogenesis. In keratinocytes, DNA methylation and demethylation regulate diabetic wound healing via modifying promoters of various factors, such as MMP-9, E2F1 and TSP1.

tory cells has demonstrated that the predominant phenotype of macrophages in the early inflammatory phase of wound healing is the M1 phenotype, which is polarized to the M2 phenotype from late inflammatory to pro-repair phase [75]. However, in diabetic wounds environment, macrophage phenotype polarization is aberrant, and the ability of macrophages to switch from the pro-inflammatory M1 phenotype to the pro-repair M2 phenotype is severely compromised, thus leading to impaired diabetic wound healing [76,77]. Interestingly, macrophage expression is regulated by DNA methylation and affects diabetic wound healing [78]. Specifically, by analyzing a model of diabetic hindlimb ischemia with whole-genome DNA methylation sequencing, Bahu et al. found that the promoter of the pro-inflammatory M1 gene was hypomethylated in diabetic ischemic muscle, whereas the promoter of the anti-inflammatory, pro-angiogenic M2 gene was hypermethylated [79]. This further suggests that abnormal DNA methylation status in patients with DFU results in more pro-inflammatory M1 phenotypes than anti-inflammatory, pro-angiogenic and tissue repair M2 phenotypes in diabetic damaged tissues, which leads to diminished angiogenesis, impaired tissue repair in the diabetic state and delayed wound healing.

DNA methylation also has a role in regulating the differentiation of hematopoietic stem cells into macrophages. As a member of the nitrogen oxide family of NADPH oxidases, NOX-2 contributes significantly to reactive oxygen species (ROS) production, whose overexpression causes oxidative stress in tissue cells and can have a negative impact on wound healing [80]. For example, Yan and co-authors found that increased oxidative stress induced by *NOX-2* in bone marrow-derived hematopoietic stem cells (HSCs) of diabetic mice suppressed microRNA let-7d-3p, which in turn directly upregulated DNMT1, leading to DNA hypermethylation in *Notch1*, *PU.1* and kruppel-like factor (*Klf4*) and hence downregulated expression of these genes in HSCs (Fig. 3; Table 1) [81]. As a widespread classical pathway, Notch signaling is closely associated with the regulation of the differentiation and development of cells, tissues and organs. Particularly, *Notch1* was found to take a leading role in wound healing and in the recruitment of macrophages with both M1 and M2 phenotypes [82]. *PU.1* is a transcription factor, which can determine macrophages' fate by binding to their regulatory regions [83,84]. As a downstream target gene of *PU.1* and *Notch 1*, *Klf4* regulates the differentiation of bone marrow-derived HSCs into mononuclear macrophages, promotes macrophage M2 polarization, and suppresses the M1 phenotype. This is justified by the fact that *Klf4* deficiency in bone marrow cells leads to delayed wound healing and increased M1 macrophages[85,86]. Taken

Table 1
Impacts of DNA methylation and demethylation on diabetic wound healing and the involved genes under specific diabetic conditions.

Factor	Diabetic condition	Cell lines	DNA methylation/ demethylation status ()	Related genes	Impacts	References (PMID)
NA	diabetic ischemic muscle	M1 M2	Methylation (↓) Methylation (↑)	*Cfb*, *Serping1*, *Tnfsf15* *Nrp1*, *Cxcr4*, *Plxnd1*, *Arg1*, *Cdk18*, *Fes*	Delayed wound healing	26,085,133 [79]
NA	Patients with DFU	NA	Methylation (↑)	*TLR2*	Poor diabetic wound healing	25,541,252 [89]
DNMT1	T2DM, Oxidant stress	HSCs	Methylation (↑)	*Nox-2*, *Notch1*, *miR-let-7d-3p*, *PU.1*, *Klf4*	Increased M1 macrophages Delayed wound healing	29,295,997 [81]
	Transient high glucose	HUVECs	Methylation (↑)	*Ang-1/NF-κB*	Endothelial cell dysfunction	33,259,831 [97]
	Nitric oxide	MSCs	Methylation (↓)	*Flt1*	Endothelial differentiation	30,997,675 [99]
	T1DM, Hydrogen sulfide	HUVECs	Methylation (↓)	*miR-126-3p*	Improved angiogenesis	36,078,059 [100]
	Wound-edge from patients with DFU	HaCaT	Methylation (↑)	*TGF-β*, *WAKMAR1*, *E2F1*	Impaired re-epithelialization	31,019,085 [119]
NA	Type I diabetic rat model	NA	Methylation (↓)	*Alu*	Genomic instability Poor wound healing	35,127,718 [130]
NA	High-glucose, Decitabin	HaCaT	Methylation (↓)	*TSP1*	Impaired angiogenesis	26,678,678 [121]
TET2	Healthy tissue	SMCs	Demethylation (↑)	*mTORC1*, *MYOCD*, *SRF*, *MYH11*	Improved vascular repair	24,077,167 [103]
	Ascorbic acid 2-glucoside	BMSCs	Demethylation (↑)	*PI3K/AKT*	Promoted angiogenesis Accelerated wound healing	35,313,962 [104]
	AGEs	HaCaT	Demethylation (↑)	*MMP-9*	Imbalance between ECM synthesis and degradation	26,913,994 [112]
	Skin, wound fluids	HaCaT	Demethylation (↑)	*MMP-9*	Imbalance between ECM synthesis and degradation	26,921,880 [113]
	AGEs	HaCaT	Demethylation (↑)	*TETILA*, *MMP-9*	Imbalance between ECM synthesis and degradation	31,653,825 [117]
NA	AGEs	HaCaT	Demethylation (↑)	*Ras / ERK*, *MMP-9*	Imbalance between ECM synthesis and degradation	25,916,956 [114]
NA	TNF-α, Decitabine	HaCaT	Demethylation (↑)	*MMP-9*	Imbalance between ECM synthesis and degradation	23,417,766 [116]
TDG	AGEs	HaCaT	Demethylation (↑)	*GADD45a*, *MMP-9*	Imbalance between ECM synthesis and degradation	29,244,109 [115]

NA, Not available; AGEs, Advanced glycation end products; ECM, Extracellular matrix; T2DM, type 2 diabetes mellitus; *Cfb*, Complement Factor B; *Tnfsf15*, Tumor Necrosis Factor Ligand Superfamily Member 15; *Nrp1*, *Neuronilin-1*; *Cxcr4*, CXC receptor 4; *Plxnd1*, Plexin D1; *Arg1*, Arginase 1; *Cdk18*, Cyclin-dependent kinase-18; *TLR2*, Toll-like receptor 2; *Klf4*, Kruppel-like factor; *SRF*, Serum response factor; *TSP1*, Thrombospondin-1, *TNF-α*, Tumor necrosis factor-α; TDG, Thymidine-DNA glycosylase.

together, targeting and regulating DNA methylation and macrophage polarization can promote diabetic wound healing.

DNA methylation and role of TLR2 in diabetic wound healing

Toll-like receptor 2 (TLR2) belongs to the mammalian toll family of leucine-rich proteins, mainly functions to regulate immune cells, including monocytes and granulocytes among others [87]. TLR2 has been demonstrated to promote endothelial cell migration, angiogenesis and wound healing [88]. By analyzing the methylation status of the 5′-proximal region of the human *TLR2* gene, Singh et al. found that most patients with DFU had complete or partial methylation of the CpG site of the *TLR2* gene promoter, suggesting that DNA methylation of the *TLR2* promoter may lead to downregulation of TLR2, which in turn causes poor wound healing in diabetic patients (Fig. 3) [89]. In contrast, Dasu et al. concluded that increased expression of TLR2 in diabetic wounds would lead to excessive wound inflammation, whereas lack of TLR2 might reduce inflammation and promote wound healing [90]. This is consistent with the previous finding that TLR2 expression levels increase with the severity of diabetic wounds [91]. Notably, TLR2 plays different roles in different stages of diabetic wound healing. Specifically, in the inflammatory phase, TLR2 is downregulated to improve inflammation, due to full or partial methylation of the CpG site in the *TLR2* promoter. In contrast, during the angiogenic phase, TLR2 downregulation impairs endothelial cell migration and angiogenesis as TLR2 methylation persists.

DNA methylation and demethylation mediate angiogenesis in diabetic wound healing

An important cause of poor wound healing in diabetes is impaired angiogenesis. DNMT1 plays a key role in angiogenesis, and several studies have shown that inhibition of DNMT1 expression is beneficial for angiogenesis [92,93]. Angiopoietin-1 (Ang-1) and angiopoietin-2 (Ang-2) are key regulators of angiogenesis and maintenance of vascular stability, which promote the formation of mature and functional microvessels and maintain endothelial integrity [94]. In diabetic mice wounds, the expression of Ang-1 and Ang-2 is dysregulated, and the imbalance of Ang-1 and Ang-2 regulation is associated with vascular dysfunction in diabetic patients [95,96]. Particularly, Zhao et al. discovered that DNMT1 expression and activity were increased in vascular ECs after transient hyperglycemia and that elevated DNMT1 led to *Ang-1* hypermethylation and downregulation of Ang-1 expression, which in turn resulted in sustained activation of nuclear factor-κB (*NF-κB*) and subsequent endothelial dysfunction [97]. In vivo studies have further shown that inhibition of DNMT1 can regulate the Ang-1/NF-κB signaling pathway to promote angiogenesis and accelerate diabetic wound healing [97]. Nitric oxide (NO) has been extensively studied as a gaseous drug commonly used in the treatment of diabetic wound healing [98]. A study by Bandara found that NO was able to inhibit DNMT1 expression, thereby inhibiting VEGF receptor *Flt-1* promoter methylation and promoting the differentiation of mesenchymal stem cells to endothelial cells [99]. A recent

study of hydrogen sulfide (H_2S), an emerging gaseous drug to promote wound healing, found that H_2S was able to downregulate high glucose-induced DNMT1 while promoting *miR-126-3p* expression and improving angiogenesis in diabetic mice [100]. These results emphasize the long-term effects that hyperglycemia-induced DNA methylation has on poor wound healing, and advise that targeting DNMT1 has therapeutic potential in the treatment of poor diabetic wound healing.

In the wound healing process, smooth muscle cells (SMCs) have potential capabilities in pro-angiogenic and wound healing [101,102]. TET2 and 5hmc are high in mouse and human vascular mature SMCs, whereas their expression is decreased in response to vascular injuries. TET2, whose expression is negatively regulated by mTORC1, binds and modify CArG-rich regions of active SMCs' contractile promoters (*MYOCD*, *SRF*, and *MYH11*), thus enhancing SMCs' differentiation and improving vascular repair [103].

A recent study found that the antioxidant ascorbic acid 2-glucoside (AA2G) could enhance the demethylation process of bone marrow mesenchymal stem cells (BMSCs) by activating the PI3K/AKT signaling pathway and promoting TET2 expression, which in turn promotes the angiogenic capacity of BMSCs and accelerates wound healing [104]. Notably, sustained hyperglycemia in a high-glucose environment destabilizes TET2 and reduces 5hmc levels [105]. This is thought to originate from the inhibition of AMPK in a high-glucose environment and the inability of serine 99 (S99) in TET2 to be activated by AMPK phosphorylation, which protects TET2 from calpain-mediated degradation [105]. In terms of angiogenesis, AMPK activation has a bidirectional role in angiogenesis: on one hand, AMPK attenuates PI3K/Akt/mTOR-induced angiogenesis; on the other hand, AMPK activation mediates the stress response to promote autophagy, thereby stabilizing HIF-1α and thus increasing VEGF expression [106]. Interestingly, PI3K/AKT is also able to activate HIF-1α, which promotes proliferation and migration of adipose-derived stem cells (ADSCs), furthermore ADSC-induced angiogenesis in human umbilical vein endothelial cells (HUVECs) [107]. In sum, wound healing can be promoted by improving DNA demethylation process in bone marrow mesenchymal stem cells (BMSCs), which can be achieved by upregulating or stabilizing TET2 and by activating PI3K/AKT signaling pathway.

DNA methylation and demethylation in fibroblasts and keratinocytes in diabetic wound healing

In a genome-wide DNA methylation profile of foot fibroblast cell lines from patients with DFU, DNA methylation was significantly reduced in foot fibroblasts from patients with DFU than in those from non-diabetic patients and diabetic non-ulcer patients [108]. Particularly, differential methylation of several genes related to redox, myogenic fiber contraction, angiogenesis and ECM function was identified [108]. It was found that: i) DNA hypermethylation was more common than DNA hypomethylation at chronic wound margins; ii) downregulation of DNA hypermethylated genes inhibited epithelial-mesenchymal transition and impaired wound healing; and that iii) correction of DNA hypermethylation was effective in improving wound closure [72]. This demonstrates that targeting DNA hypermethylation has a therapeutic potential in promoting wound healing.

Keratinocytes are the primary cells in the epidermis, which are important in the wound healing process, especially in re-epithelialization. Matrix metalloproteinase-9 (MMP-9), a type IV collagenase expressed by keratinocyte at the wound's leading edge, controls wound healing by altering the wound matrix and enabling cell migration and tissue remodeling. Notably, the effect of MMP-9 on wound healing is bidirectional; epithelialization is delayed when normal levels of MMP-9 expression are inhibited, and healing is impaired when MMP-9 is overexpressed [109]. In diabetic patients, MMP-9 is highly expressed in keratinocytes, leading to impaired epithelialization, and consequently impaired diabetic wound healing [110]. High expression of MMP-9 is mainly produced by the induction of advanced glycosylation end products (AGEs) [111]. In human primary keratinocytes, AGEs upregulate TET2 gene expression, thus making DNA demethylation upregulated in specific regions of the *MMP-9* promoter and elevating the expression level of MMP-9 [112]. This agrees with the fact that TET2 expression is significantly higher in epidermal cells of diabetic patients than those in normal skin. Interestingly, the tricarboxylic acid (TCA) cycle metabolite α-ketoglutarate (α-KG) is an important cofactor in regulating TET2 regulation, whose elevation is associated with local hypoxia, ischemic states and poor systemic glycemic control [113]. This suggests that DNA demethylation and wound healing may be also regulated by cell metabolism.

Various CpG sites were identified in *MMP-9*, and their methylation and demethylation status were reported to mediate the activity of *MMP-9*. Specifically, the methylation status of the −562 bp CpG site closely regulates the activity of *MMP-9* promoter, which can be inhibited by administration of the mevalonate pathway inhibitor simvastatin [114]. Furthermore, significantly reduced DNA methylation of three CpG sites (–233, –223 and −36 bp) were identified in *MMP-9*, which upregulated growth arrest and DNA-damage-inducible protein GADD45 alpha (GADD45a) to activate *MMP-9* transcription, through demethylation of thymidine-DNA glycosylase (TDG)-dependent BER promoter [115]. Moreover, the −36 bp site of *MMP-9* promoter was found to be the key site for demethylation of *MMP-9* promoter, which is regulated by lncRNAs, in human keratinocytes treated with tumor necrosis factor-α (TNF-α) [116]. Additionally, significant reductions in methylation were observed at three CpG sites (-712, –233 and −36 bp) of the *MMP-9* promoter during the demethylation process [117]. In the diabetic environment, the promoter of MMP-9 undergoes demethylation, is hypomethylated in keratinocytes, and its overexpression impairs the balance of ECM synthesis and degradation [117]. BER-mediated *MMP-9* promoter demethylation requires a TET2-interacting long noncoding RNA (*TETILA*), which recruits thymine-DNA glycosylase (TDG) to form a TET2-TDG-TETILA complex [117].

Apart from mediating diabetic wound healing via regulating MMP-9, DNA methylation in keratinocytes impacts diabetic wound healing by controlling the expression of other factors such as E2F1 and thrombospondin-1 (*TSP1*). For example, inhibiting promoter methylation of *E2F1* contributes to normal and timely wound healing by increasing the expression level of E2F1, which is engaged in maintaining the proliferation of epidermal keratinocytes [118]. Promoter methylation of *E2F1* can be inhibited by a skin-specific lncRNA (*LOC105372576*), named "wound and keratinocyte migration-associated LncRNA1 (*WAKMAR1*)", via isolating DNMTs [119]. *WAKMAR1* is induced by TGF-β signaling and expressed in keratinocytes, with a function of regulating keratinocytes' proliferation, adhesion and migration[120] Therefore, *WAKMAR1* can promote diabetic wound healing by inhibiting the DNA methylation of *E2F1* [119,120]. In contrast, DNA hypomethylation in the promoter region of *TSP1* in keratinocytes, induced by increased oxidative stress in a high-glucose environment, impairs wound healing via upregulating the expression of *TSP1* [121]. Overexpression of TSP1 inhibits skin tissue repair and granulation tissue formation, by impairing fibroblasts' migration and wound angiogenesis [122]. Early and timely administration of antioxidants normalizes TSP1 expression and the overall DNA methylation status in the skin of diabetic rats, further consequently improves wound healing in vivo [121].

Aberrant DNA methylation in other proteins involved in wound healing

In the innate immune response to tissue injury, complement activation is a crucial effector mechanism. Previous studies have mainly focused on its activation being beneficial to wounds [123,124]. Following negative pressure wound therapy (NPWT) for DFU, genes for proteins that play a major part in complement system activation, such as complement protein 2 (C2), complement protein 3 (C3), complement protein 4A (C4A) and complement protein 4B (C4B), were found to be hypermethylated. This suggests that DNA hypermethylation of complement proteins inhibits the complement system and may promote diabetic wound healing [125]. However, it has recently been found that sustained activation of the complement system in chronic wounds causes delayed wound healing [126,127]. Therefore, activation of the complement system promotes wound healing, but excessive activation can delay wound healing.

The Alu element in humans (B1 element in rodents) is a member of the short retrotransposon (SINE) family in the mammalian genome, which is located in a noncoding region. *Alu* is predominantly methylated as *Alu* (*B1*) methylation, forming heterochromatin to maintain genomic stability [128]. In diabetes, Alu hypomethylation levels are significant and are associated with hypertension, a degenerative disease of aging [129,130]. Administration of Alu siRNA increases Alu element methylation and prevents DNA damage [129,130]. Notably, use of B1 siRNA not only restored B1 methylation status and improved genomic stability, but also promoted wound healing in diabetes [131]. This suggests that genomic instability caused by Alu element hypomethylation also leads to poor diabetic wound healing, and can be targeted for improving diabetic wound healing.

Cellular reprogramming and epigenetic remodeling in diabetic wound healing

The process of poor diabetic wound healing is associated with abnormal cellular progression. In recent years, cellular reprogramming has attracted more and more attention of researchers in the field of diabetic wound healing. During cellular reprogramming, epigenetic alterations caused by the disease state are simultaneously eliminated, which helps normalize their cellular phenotype and broadens research ideas to develop therapeutics for promoting diabetic skin wound healing [132,133]. Cell reprogramming techniques have been explored mostly in somatic cell reprogramming induced multifunctional stem cells (iPSCs). Senescent fibroblasts can be reprogrammed into iPSCs and differentiated into young fibroblasts to regulate wound healing [134]. For example, Kaspur and his colleagues improved diabetic wound healing by generating iPSCs from primary DFU-derived fibroblasts and then differentiating them into fibroblasts [135]. In addition, through cellular reprogramming, the epigenetics of the re-differentiated fibroblasts can be altered, eliminating the miRNA-mediated epigenetics of poor diabetic healing [133].

Since reprogrammed iPSCs often lose somatic cell properties, Gill and his team developed the "maturation transient reprogramming technique (MPTR)", which terminates fibroblasts before they are reprogrammed into stem cells, allowing the cells to maintain fibroblast function [136]. In addition to reprogramming to iPSCs for re-differentiation, somatic cells can be directly reprogrammed into healing-associated cells. For instance, Kurita et al. promoted the regeneration of skin ulcer surfaces by directly reprogramming traumatic mesenchymal stem cells (MSCs) to epithelial cells, to re-epithelialize all areas of the wound [137]. Moreover, MSCs to ECs and reprogramming ECs enhance the neovascularization of diabetic wounds and accelerate wound healing [138]. Additionally, through induction, M1 macrophages can be directly reprogrammed into M2 macrophages, thus promoting fibroblast proliferation, migration and endothelial cell vascularization to improve diabetic wound healing [139,140].

DNA methylation, which is the most stable epigenetic alteration, is a significant obstacle to iPSC cell reprogramming. The efficiency of iPSC cell generation can be improved by the demethylating agents 5-azacytidine and demethylase [141]. Moreover, although the epigenetic memory is reset after reprogramming cells into iPSC, the DNA methylation profile of their parent cells remains in the reprogrammed iPSC, and the residual methylation profile affects the function of the re-differentiated cells [142]. By controlling DNA methylation status, Katz et al. reprogrammed fibroblasts into islet-like cells, which were able to secrete insulin in response to glucose from human dermal fibroblasts [143]. In sum, we propose that DNA methylation regulates cellular reprogramming, and can be targeted to promote diabetic wound healing.

Conclusion

Diabetes is becoming more and more prevalent in the world. Diabetic patients suffer numerous complications, among which DFU is one of the most common and serious. DFU can give rise to limb amputation or even death, if it is not treated well. This imposes huge financial and health burden on patients with DFU worldwide. Hence, there is an urgent need for the development of therapeutics to improve the challenging treatment of DFU. This review provides an overview of the pathobiological and molecular mechanisms of DFU, focusing on the abnormality of DFU in the four main stages of wound healing, in-depth regulation of DNA methylation and demethylation in DFU, as well as the cellular reprogramming in DFU.

Disappointingly, diabetic patients fail to pass through the four phases of wound healing and undergo impaired wound healing, due to the prolonged inflammatory phase of skin wounds, abnormal growth factor secretion, impaired ECM, impaired microvascular function and angiogenesis, and impaired epithelialization and remodeling. Under diabetic conditions, DNMT1 and TET2 are often upregulated to methylate and demethylated gene promoters respectively, which mediates their protein expression levels and consequently causes poor wound healing. Moderate expression of proteins is often beneficial for wound healing, while excessive or lack of expression of key proteins may impair diabetic wound healing. Upregulation of DNMT1 and TET2 were found in different cells under different diabetic wound conditions, which regulates different signally pathways by methylating/demethylating corresponding genes (Table 1). Therefore, targeted drugs can be developed to improve the treatment of DFU, by regulating the DNA methylation and demethylation activity of DNMT1 and TET2, and by normalizing the expression levels of DNMT, TET2 and the corresponding proteins that cause or are affected by the abnormal DNA methylation and demethylation. In the past few years, the medical field has increasingly focused on predictive, preventive and personalized medicine (PPPM) strategies through which we can effectively reduce the incidence of disease and improve the quality of life of patients [144–147]. DNA methylation has been used in preclinical trials to screen for cervical lesions (ClinicalTrials.gov Identifier: NCT03960879), advanced colorectal adenomatous polyps and cancers (ClinicalTrials.gov Identifier: NCT04221854), but DNA methylation has been used in wound healing for a number of reasons. methylation in wound healing has not been studied.

This work highlights the importance of epigenetic, particularly DNA methylation and demethylation modifications, in diabetic

wound healing, and provides precious insights into the development therapeutics targeting diabetic wound healing. Given that regulation of DNA methylation and demethylation varies significantly among different types of cells under different diabetic conditions, it is challenging to uncover the molecular-level mechanisms that define how abnormality in DNA methylation status impact diabetic wound healing. Urgent research is quired to overcome this challenge for the improvement of the DFU treatment.

Compliance with ethics requirements

This review does not contain any studies with human or animal subjects.

CRediT authorship contribution statement

Jun-Yu Deng: Conceptualization, Writing – original draft. **Xing-Qian Wu:** Investigation. **Wen-Jie He:** Investigation. **Xin Liao:** Visualization. **Ming Tang:** Writing – review & editing. **Xu-Qiang Nie:** Writing – review & editing, Funding acquisition, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

[1] Sun H, Saeedi P, Karuranga S, Pinkepank M, Ogurtsova K, Duncan BB, et al. Idf diabetes atlas: Global, regional and country-level diabetes prevalence estimates for 2021 and projections for 2045. Diabetes Res Clin Pract 2022;183:109–19.
[2] Armstrong DG, Boulton AJM, Bus SA. Diabetic foot ulcers and their recurrence. N Engl J Med 2017;376(24):2367–75.
[3] Lo ZJ, Surendra NK, Saxena A, Car J. Clinical and economic burden of diabetic foot ulcers: A 5-year longitudinal multi-ethnic cohort study from the tropics. Int Wound J 2021;18(3):375–86.
[4] Natarajan R. Epigenetic mechanisms in diabetic vascular complications and metabolic memory: The 2020 edwin bierman award lecture. Diabetes 2021;70(2):328–37.
[5] Boyko EJ, Zelnick LR, Braffett BH, Pop-Busui R, Cowie CC, Lorenzi GM, et al. Risk of foot ulcer and lower-extremity amputation among participants in the diabetes control and complications trial/epidemiology of diabetes interventions and complications study. Diabetes Care 2022;45(2):357–64.
[6] Zhang H-H, Han X, Wang M, Hu Q, Li S, Wang M, et al. The association between genomic DNA methylation and diabetic peripheral neuropathy in patients with type 2 diabetes mellitus. J Diabetes Res 2019; 2019:2494057-2494057https://doi.org/10.1155/2019/2494057.
[7] Guo K, Elzinga S, Eid S, Figueroa-Romero C, Hinder LM, Pacut C, et al. Genome-wide DNA methylation profiling of human diabetic peripheral neuropathy in subjects with type 2 diabetes mellitus. Epigenetics 2019;14(8):766–79.
[8] Ding GL, Huang HF. Role for tet in hyperglycemia-induced demethylation: A novel mechanism of diabetic metabolic memory. Diabetes 2014;63(9):2906–8.
[9] Dubey R, Prabhakar PK, Gupta J. Epigenetics: Key to improve delayed wound healing in type 2 diabetes. Mol Cell Biochem 2022;477(2):371–83.
[10] Ahmed SAH, Ansari SA, Mensah-Brown EPK, Emerald BS. The role of DNA methylation in the pathogenesis of type 2 diabetes mellitus. Clin Epigenetics 2020;12(1):104.
[11] Li X, Weber NC, Cohn DM, Hollmann MW, DeVries JH, Hermanides J, et al. Effects of hyperglycemia and diabetes mellitus on coagulation and hemostasis. J Clin Med 2021;10(11).
[12] Martin P, Leibovich SJ. Inflammatory cells during wound repair: The good, the bad and the ugly. Trends Cell Biol 2005;15(11):599–607.
[13] Wolf SJ, Melvin WJ, Gallagher K. Macrophage-mediated inflammation in diabetic wound repair. Semin Cell Dev Biol 2021;119:111–8.
[14] Mu X, Wu X, He W, Liu Y, Wu F, Nie X. Pyroptosis and inflammasomes in diabetic wound healing. Front Endocrinol (Lausanne) 2022;13:950798.
[15] Wilkinson HN, Hardman MJ. Wound healing: Cellular mechanisms and pathological outcomes. Open Biol 2020;10(9):200–23.
[16] Swoboda L, Held J. Impaired wound healing in diabetes. J Wound Care 2022;31(10):882–5.
[17] Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: A cellular perspective. Physiol Rev 2019;99(1):665–706.
[18] Veves A. Repair, regeneration and the future. J Wound Care 2020; 29(10): 539-539https://doi.org/10.12968/jowc.2020.29.10.539.
[19] Zhao R, Liang H, Clarke E, Jackson C, Xue M. Inflammation in chronic wounds. Int J Mol Sci 2016;17(12):2085.
[20] Kim SY, Nair MG. Macrophages in wound healing: Activation and plasticity. Immunol Cell Biol 2019;97(3):258–67.
[21] Aitcheson SM, Frentiu FD, Hurn SE, Edwards K, Murray RZ. Skin wound healing: Normal macrophage function and macrophage dysfunction in diabetic wounds. Molecules 2021;26(16):4917.
[22] Sun JX, Xu XH, Jin L. Effects of metabolism on macrophage polarization under different disease backgrounds. Front Immunol 2022;13:880286.
[23] Munoz J, Akhavan NS, Mullins AP, Arjmandi BH. Macrophage polarization and osteoporosis: A review. Nutrients 2020;12(10):2999.
[24] Fu J, Huang J, Lin M, Xie T, You T. Quercetin promotes diabetic wound healing via switching macrophages from m1 to m2 polarization. J Surg Res 2020;246:213–23.
[25] Li M, Hou Q, Zhong L, Zhao Y, Fu X. Macrophage related chronic inflammation in non-healing wounds. Front Immunol 2021;12:681–710.
[26] Huang J, Zhang S, Ding X, Li S, Luo X, Cao Y, et al. Research progress on the mechanism by which skin macrophage dysfunction mediates chronic inflammatory injury in diabetic skin. Front Endocrinol (Lausanne) 2022;13:960551.
[27] Atkin-Smith GK. Phagocytic clearance of apoptotic, necrotic, necroptotic and pyroptotic cells. Biochem Soc Trans 2021;49(2):793–804.
[28] Rousselle P, Montmasson M, Garnier C. Extracellular matrix contribution to skin wound re-epithelialization. Matrix Biol 2019;75–76:12–26.
[29] Amiri N, Golin AP, Jalili RB, Ghahary A. Roles of cutaneous cell-cell communication in wound healing outcome: An emphasis on keratinocyte-fibroblast crosstalk. Exp Dermatol 2022;31(4):475–84.
[30] Veith A P, Henderson K, Spencer A, Sligar A D, Baker A B. Therapeutic strategies for enhancing angiogenesis in wound healing. Adv Drug Deliv Rev 2019; 14697-125https://doi.org/10.1016/j.addr.2018.09.010.
[31] Cui L, Liang J, Liu H, Zhang K, Li J. Nanomaterials for angiogenesis in skin tissue engineering. Tissue Eng Part B Rev Part B, Reviews 2020;26(3):203–16.
[32] Okonkwo UA, DiPietro LA. Diabetes and wound angiogenesis. Int J Mol Sci 2017;18(7):14–9.
[33] Xiao T, Yan Z, Xiao S, Xia Y. Proinflammatory cytokines regulate epidermal stem cells in wound epithelialization. Stem Cell Res Ther 2020;11(1):232.
[34] Rousselle P, Braye F, Dayan G. Re-epithelialization of adult skin wounds: Cellular mechanisms and therapeutic strategies. Adv Drug Deliv Rev 2019;146:344–65.
[35] Hight-Warburton W, Felix R, Burton A, Maple H, Chegkazi MS, Steiner RA, et al. A4/α9 integrins coordinate epithelial cell migration through local suppression of map kinase signaling pathways. Front Cell Dev Biol 2021;9:750–71.
[36] Piipponen M, Li D, Landén NX. The immune functions of keratinocytes in skin wound healing. Int J Mol Sci 2020;21(22):87–90.
[37] Qu H, Miao T, Wang Y, Tan L, Huang B, Zhang L, et al. Dedicator of cytokinesis 5 regulates keratinocyte function and promotes diabetic wound healing. Diabetes 2021;70(5):1170–84.
[38] Hosseini MN. The role of keratinocyte function on the defected diabetic wound healing. Int J Burns Trauma 2021;11(6):430–41.
[39] Abbasi S, Sinha S, Labit E, Rosin NL, Yoon G, Rahmani W, et al. Distinct regulatory programs control the latent regenerative potential of dermal fibroblasts during wound healing. Cell Stem Cell 2020;27(3):396–412.e6.
[40] Foster DS, Januszyk M, Yost KE, Chinta MS, Gulati GS, Nguyen AT, et al. Integrated spatial multiomics reveals fibroblast fate during tissue repair. Proc Natl Acad Sci U S A 2021;118(41).
[41] Monika P, Waiker PV, Chandraprabha MN, Rangarajan A, Murthy KNC. Myofibroblast progeny in wound biology and wound healing studies. Wound Repair Regen 2021;29(4):531–47.
[42] Shook BA, Wasko RR, Rivera-Gonzalez GC, Salazar-Gatzimas E, Lopez-Giraldez F, Dash BC, et al. Myofibroblast proliferation and heterogeneity are supported by macrophages during skin repair. Science 2018;362(6417): eaar2971.
[43] Liu Y, Liu Y, He W, Mu X, Wu X, Deng J, et al. Fibroblasts: Immunomodulatory factors in refractory diabetic wound healing. Front Immunol 2022;13:918223.
[44] Wan R, Weissman JP, Grundman K, Lang L, Grybowski DJ, Galiano RD. Diabetic wound healing: The impact of diabetes on myofibroblast activity and its potential therapeutic treatments. Wound Repair Regen 2021;29(4):573–81.
[45] Stunova A, Vistejnova L. Dermal fibroblasts-a heterogeneous population with regulatory function in wound healing. Cytokine Growth Factor Rev 2018;39:137–50.

[46] El Ayadi A, Jay JW, Prasai A. Current approaches targeting the wound healing phases to attenuate fibrosis and scarring. Int J Mol Sci 2020;21(3):1105.
[47] Kandhwal M, Behl T, Singh S, Sharma N, Arora S, Bhatia S, et al. Role of matrix metalloproteinase in wound healing. Am J Transl Res 2022;14(7):4391–405.
[48] Komi DEA, Khomtchouk K, Santa Maria PL. A review of the contribution of mast cells in wound healing: Involved molecular and cellular mechanisms. Clin Rev Allergy Immunol 2020;58(3):298–312.
[49] Theocharidis G, Baltzis D, Roustit M, Tellechea A, Dangwal S, Khetani RS, et al. Integrated skin transcriptomics and serum multiplex assays reveal novel mechanisms of wound healing in diabetic foot ulcers. Diabetes 2020;69 (10):2157–69.
[50] Theocharidis G, Thomas BE, Sarkar D, Mumme HL, Pilcher WJR, Dwivedi B, et al. Single cell transcriptomic landscape of diabetic foot ulcers. Nat Commun 2022;13(1):181.
[51] Chen Z, Zhang Y. Role of mammalian DNA methyltransferases in development. Annu Rev Biochem 2020;89:135–58.
[52] Goll MG, Bestor TH. Eukaryotic cytosine methyltransferases. Annu Rev Biochem 2005;74(1):481–514.
[53] Edwards JR, Yarychkivska O, Boulard M, Bestor TH. DNA methylation and DNA methyltransferases. Epigenetics Chromatin 2017; 1023..
[54] Chen ZX, Riggs AD. DNA methylation and demethylation in mammals. J Biol Chem 2011;286(21):18347–53.
[55] Okano M, Bell DW, Haber DA, Li E. DNA methyltransferases dnmt3a and dnmt3b are essential for de novo methylation and mammalian development. Cell 1999;99(3):247–57.
[56] Singh AK, Zhao B, Liu X, Wang X, Li H, Qin H, et al. Selective targeting of tet catalytic domain promotes somatic cell reprogramming. Proc Natl Acad Sci U S A 2020;117(7):3621–6.
[57] Li W, Xu L. Epigenetic function of tet family, 5-methylcytosine, and 5-hydroxymethylcytosine in hematologic malignancies. Oncol Res Treat 2019;42(6):309–18.
[58] Wang D, Wu W, Callen E, Pavani R, Zolnerowich N, Kodali S, et al. Active DNA demethylation promotes cell fate specification and the DNA damage response. Science 2022;378(6623):983–9.
[59] Stoyanova E, Riad M, Rao A, Heintz N. 5-hydroxymethylcytosine-mediated active demethylation is required for mammalian neuronal differentiation and function. Elife 2021;10:e66973.
[60] Wu H, Zhang Y. Reversing DNA methylation: Mechanisms, genomics, and biological functions. Cell 2014;156(1–2):45–68.
[61] Tsagaratou A, Lio CJ, Yue X, Rao A. Tet methylcytosine oxidases in t cell and b cell development and function. Front Immunol 2017;8220.
[62] De Riso G, Fiorillo DFG, Fierro A, Cuomo M, Chiariotti L, Miele G, et al. Modeling DNA methylation profiles through a dynamic equilibrium between methylation and demethylation. Biomolecules 2020;10(9):1271.
[63] Mayer W, Niveleau A, Walter J, Fundele R, Haaf T. Demethylation of the zygotic paternal genome. Nature 2000; 403(6769): 501-502https://doi.org/10.1038/35000656.
[64] Greenberg MVC, Bourc'his D. The diverse roles of DNA methylation in mammalian development and disease. Nat Rev Mol Cell Biol 2019;20 (10):590–607.
[65] Davison GW, Irwin RE, Walsh CP. The metabolic-epigenetic nexus in type 2 diabetes mellitus. Free Radic Biol Med 2021;170:194–206.
[66] Pinzon-Cortes JA, Perna-Chaux A, Rojas-Villamizar NS, Diaz-Basabe A, Polania-Villanueva DC, Jacome MF, et al. Effect of diabetes status and hyperglycemia on global DNA methylation and hydroxymethylation. Endocr Connect 2017;6(8):708–25.
[67] Stenvinkel P, Karimi M, Johansson S, Axelsson J, Suliman M, Lindholm B, et al. Impact of inflammation on epigenetic DNA methylation ? A novel risk factor for cardiovascular disease? J Intern Med 2007;261(5):488–99.
[68] Ye J, Stefan-Lifshitz M, Tomer Y. Genetic and environmental factors regulate the type 1 diabetes gene ctsh via differential DNA methylation. J Biol Chem 2021;296100774.
[69] Kindt ASD, Fuerst RW, Knoop J, Laimighofer M, Telieps T, Hippich M, et al. Allele-specific methylation of type 1 diabetes susceptibility genes. J Autoimmun 2018:8963–74.
[70] Pastar I, Marjanovic J, Stone RC, Chen V, Burgess JL, Mervis JS, et al. Epigenetic regulation of cellular functions in wound healing. Exp Dermatol 2021;30 (8):1073–89.
[71] Lewis CJ, Stevenson A, Fear MW, Wood FM. A review of epigenetic regulation in wound healing: Implications for the future of wound care. Wound Repair Regen 2020;28(6):710–8.
[72] Singh K, Rustagi Y, Abouhashem AS, Tabasum S, Verma P, Hernandez E, et al. Genome-wide DNA hypermethylation opposes healing in chronic wound patients by impairing epithelial-to-mesenchymal transition. J Clin Invest 2022;132(17):e157279.
[73] den Dekker A, Davis FM, Kunkel SL, Gallagher KA. Targeting epigenetic mechanisms in diabetic wound healing. Transl Res 2019:20439–50.
[74] Olsen A S, Sarras M P, Jr., Leontovich A, Intine R V. Heritable transmission of diabetic metabolic memory in zebrafish correlates with DNA hypomethylation and aberrant gene expression. Diabetes 2012; 61(2): 485-91https://doi.org/10.2337/db11-0588
[75] Daley JM, Brancato SK, Thomay AA, Reichner JS, Albina JE. The phenotype of murine wound macrophages. J Leukoc Biol 2010;87(1):59–67.
[76] Barman PK, Koh TJ. Macrophage dysregulation and impaired skin wound healing in diabetes. Front Cell. Dev Biol 2020;8528.
[77] Knipper JA, Ding X, Eming SA. Diabetes impedes the epigenetic switch of macrophages into repair mode. Immunity 2019;51(2):199–201.
[78] Davis FM, Gallagher KA. Epigenetic mechanisms in monocytes/macrophages regulate inflammation in cardiometabolic and vascular disease. Arterioscler Thromb Vasc Biol 2019;39(4):623–34.
[79] Babu M, Durga Devi T, Makinen P, Kaikkonen M, Lesch HP, Junttila S, et al. Differential promoter methylation of macrophage genes is associated with impaired vascular growth in ischemic muscles of hyperlipidemic and type 2 diabetic mice: Genome-wide promoter methylation study. Circ Res 2015;117 (3):289–99.
[80] Begum R, Thota S, Abdulkadir A, Kaur G, Bagam P, Batra S. Nadph oxidase family proteins: Signaling dynamics to disease management. Cell Mol Immunol 2022;19(6):660–86.
[81] Yan J, Tie G, Wang S, Tutto A, DeMarco N, Khair L, et al. Diabetes impairs wound healing by dnmt1-dependent dysregulation of hematopoietic stem cells differentiation towards macrophages. Nat Commun 2018;9(1):33.
[82] Hamilton Outtz H, Wu JK, Wang X, Kitajewski J. Notch1 deficiency results in decreased inflammation during wound healing and regulates vascular endothelial growth factor receptor-1 and inflammatory cytokine expression in macrophages. J Immunol 2010;185(7):4363–73.
[83] Natoli G. Maintaining cell identity through global control of genomic organization. Immunity 2010;33(1):12–24.
[84] Natoli G, Ghisletti S, Barozzi I. The genomic landscapes of inflammation. Genes Dev 2011;25(2):101–6.
[85] Jurkin J, Krump C, Köffel R, Fieber C, Schuster C, Brunner PM, et al. Human skin dendritic cell fate is differentially regulated by the monocyte identity factor kruppel-like factor 4 during steady state and inflammation. J Allergy Clin Immunol 2017;139(6):1873–1884.e10.
[86] Bulut GB, Alencar GF, Owsiany KM, Nguyen AT, Karnewar S, Haskins RM, et al. Klf4 (kruppel-like factor 4)-dependent perivascular plasticity contributes to adipose tissue inflammation. Arterioscler Thromb Vasc Biol 2021;41 (1):284–301.
[87] Marks KE, Cho K, Stickling C, Reynolds JM. Toll-like receptor 2 in autoimmune inflammation. Immune network 2021;21(3):e18.
[88] Xu Y, Zhou Y, Lin H, Hu H, Wang Y, Xu G. Toll-like receptor 2 in promoting angiogenesis after acute ischemic injury. Int J Mol Med 2013;31(3):555–60.
[89] Singh K, Agrawal NK, Gupta SK, Mohan G, Chaturvedi S, Singh K. Genetic and epigenetic alterations in toll like receptor 2 and wound healing impairment in type 2 diabetes patients. J Diabetes Complications 2015;29(2):222–9.
[90] Dasu MR, Thangappan RK, Bourgette A, DiPietro LA, Isseroff R, Jialal I. Tlr2 expression and signaling-dependent inflammation impair wound healing in diabetic mice. Lab Invest 2010;90(11):1628–36.
[91] Dasu MR, Martin SJ. Toll-like receptor expression and signaling in human diabetic wounds. World J Diabetes 2014;5(2):219–23.
[92] Cannella V, Piccione G, Altomare R, Marino A, Di Marco P, Russotto L, et al. Differentiation and characterization of rat adipose tissue mesenchymal stem cells into endothelial-like cells. Anat Histol Embryol 2018;47(1):11–20.
[93] Shang T, Li S, Zhang Y, Lu L, Cui L, Guo FF. Hypoxia promotes differentiation of adipose-derived stem cells into endothelial cells through demethylation of ephrinb2. Stem Cell Res Ther 2019;10(1):133.
[94] Lee HJ, Hong YJ, Kim M. Angiogenesis in chronic inflammatory skin disorders. Int J Mol Sci 2021;22(21):12035.
[95] Hayashi SI, Rakugi H, Morishita R. Insight into the role of angiopoietins in ageing-associated diseases. Cells 2020;9(12):2636.
[96] Zhou H, Chen T, Li Y, You J, Deng X, Chen N, et al. Glycation of tie-2 inhibits angiopoietin-1 signaling activation and angiopoietin-1-induced angiogenesis. Int J Mol Sci 2022;23(13):7137.
[97] Zhao J, Yang S, Shu B, Chen L, Yang R, Xu Y, et al. Transient high glucose causes persistent vascular dysfunction and delayed wound healing by the dnmt1-mediated ang-1/nf-kappab pathway. J Invest Dermatol 2021;141 (6):1573–84.
[98] Malone-Povolny MJ, Maloney SE, Schoenfisch MH. Nitric oxide therapy for diabetic wound healing. Adv Healthc Mater 2019;8(12):e1801210.
[99] Bandara N, Gurusinghe S, Kong A, Mitchell G, Wang LX, Lim SY, et al. Generation of a nitric oxide signaling pathway in mesenchymal stem cells promotes endothelial lineage commitment. J Cell Physiol 2019;234 (11):20392–407.
[100] Xue W, Zhang Q, Chen Y, Zhu Y. Hydrogen sulfide improves angiogenesis by regulating the transcription of pri-mir-126 in diabetic endothelial cells. Cells 2022;11(17):2651.
[101] Gorecka J, Gao X, Fereydooni A, Dash BC, Luo J, Lee SR, et al. Induced pluripotent stem cell-derived smooth muscle cells increase angiogenesis and accelerate diabetic wound healing. Regen Med 2020;15(2):1277–93.
[102] Xie Y, Ostriker AC, Jin Y, Hu H, Sizer AJ, Peng G, et al. Lmo7 is a negative feedback regulator of transforming growth factor β signaling and fibrosis. Circulation 2019;139(5):679–93.
[103] Liu R, Jin Y, Tang WH, Qin L, Zhang X, Tellides G, et al. Ten-eleven translocation-2 (tet2) is a master regulator of smooth muscle cell plasticity. Circulation 2013;128(18):2047–57.
[104] Yi Y, Wu M, Zhou X, Xiong M, Tan Y, Yu H, et al. Ascorbic acid 2-glucoside preconditioning enhances the ability of bone marrow mesenchymal stem cells in promoting wound healing. Stem Cell Res Ther 2022;13(1):119.
[105] Wu D, Hu D, Chen H, Shi G, Fetahu IS, Wu F, et al. Glucose-regulated phosphorylation of tet2 by ampk reveals a pathway linking diabetes to cancer. Nature 2018;559(7715):637–41.

[106] Li Y, Sun R, Zou J, Ying Y, Luo Z. Dual roles of the amp-activated protein kinase pathway in angiogenesis. Cells 2019;8(7):752.
[107] Jiang W, Zhang J, Zhang X, Fan C, Huang J. Vap-plga microspheres (vap-plga) promote adipose-derived stem cells (adscs)-induced wound healing in chronic skin ulcers in mice via pi3k/akt/hif-1α pathway. Bioengineered 2021;12(2):10264–84.
[108] Park LK, Maione AG, Smith A, Gerami-Naini B, Iyer LK, Mooney DJ, et al. Genome-wide DNA methylation analysis identifies a metabolic memory profile in patient-derived diabetic foot ulcer fibroblasts. Epigenetics 2014;9 (10):1339–49.
[109] Lan B, Zhang L, Yang L, Wu J, Li N, Pan C, et al. Sustained delivery of mmp-9 sirna via thermosensitive hydrogel accelerates diabetic wound healing. J Nanobiotechnology 2021;19(1):130.
[110] Liang Y, Yang C, Lin Y, Parviz Y, Sun K, Wang W, et al. Matrix metalloproteinase 9 induces keratinocyte apoptosis through fasl/fas pathway in diabetic wound. Apoptosis 2019;24(7–8):542–51.
[111] Zhu P, Chen C, Wu D, Chen G, Tan R, Ran J. Ages-induced mmp-9 activation mediated by notch1 signaling is involved in impaired wound healing in diabetic rats. Diabetes Res Clin Pract 2022;186:109831.
[112] Zhang J, Yang C, Wang C, Liu D, Lao G, Liang Y, et al. Age-induced keratinocyte mmp-9 expression is linked to tet2-mediated cpg demethylation. Wound Repair Regen 2016;24(3):489–500.
[113] Tan Q, Wang W, Yang C, Zhang J, Sun K, Luo HC, et al. Alpha-ketoglutarate is associated with delayed wound healing in diabetes. Clin Endocrinol (Oxf) 2016;85(1):54–61.
[114] Lu W, Li J, Ren M, Zeng Y, Zhu P, Lin L, et al. Role of the mevalonate pathway in specific cpg site demethylation on ages-induced mmp9 expression and activation in keratinocytes. Mol Cell Endocrinol 2015; 411121-129https://doi.org/10.1016/j.mce.2015.04.019.
[115] Zhou L, Wang W, Yang C, Zeng T, Hu M, Wang X, et al. Gadd45a promotes active DNA demethylation of the mmp-9 promoter via base excision repair pathway in ages-treated keratinocytes and in diabetic male rat skin. Endocrinology 2018;159(2):1172–86.
[116] Ling L, Ren M, Yang C, Lao G, Chen L, Luo H, et al. Role of site-specific DNA demethylation in tnfalpha-induced mmp9 expression in keratinocytes. J Mol Endocrinol 2013;50(3):279–90.
[117] Zhou L, Ren M, Zeng T, Wang W, Wang X, Hu M, et al. Tet2-interacting long noncoding rna promotes active DNA demethylation of the mmp-9 promoter in diabetic wound healing. Cell Death Dis 2019;10(11):813.
[118] Jones SJ, Dicker AJ, Dahler AL, Saunders NA. E2f as a regulator of keratinocyte proliferation: Implications for skin tumor development. J Invest Dermatol 1997;109(2):187–93.
[119] Li D, Kular L, Vij M, Herter EK, Li X, Wang A, et al. Human skin long noncoding rna wakmar1 regulates wound healing by enhancing keratinocyte migration. Proc Natl Acad Sci U S A 2019;116(19):9443–52.
[120] D'Souza SJ, Vespa A, Murkherjee S, Maher A, Pajak A, Dagnino L. E2f–1 is essential for normal epidermal wound repair. J Biol Chem 2002;277 (12):10626–32.
[121] Lan C C, Huang S M, Wu C S, Wu C H, Chen G S. High-glucose environment increased thrombospondin-1 expression in keratinocytes via DNA hypomethylation. Transl Res 2016; 16991-101 e1-3https://doi.org/10.1016/j.trsl.2015.11.002.
[122] Streit M, Velasco P, Riccardi L, Spencer L, Brown LF, Janes L, et al. Thrombospondin-1 suppresses wound healing and granulation tissue formation in the skin of transgenic mice. Embo j 2000;19(13):3272–82.
[123] Houseright RA, Rosowski EE, Lam PY, Tauzin SJM, Mulvaney O, Dewey CN, et al. Cell type specific gene expression profiling reveals a role for complement component c3 in neutrophil responses to tissue damage. Sci Rep 2020;10(1):15716.
[124] Wang Z, Qi F, Luo H, Xu G, Wang D. Inflammatory microenvironment of skin wounds. Front Immunol 2022:13789274.
[125] Ludwig-Slomczynska AH, Borys S, Seweryn MT, Hohendorff J, Kapusta P, Kiec-Wilk B, et al. DNA methylation analysis of negative pressure therapy effect in diabetic foot ulcers. Endocr Connect 2019;8(11):1474–82.
[126] van de Goot F, Krijnen PAJ, Begieneman MPV, Ulrich MMW, Middelkoop E, Niessen HWM. Acute inflammation is persistent locally in burn wounds: A pivotal role for complement and c-reactive protein. J Burn Care Res 2009;30 (2):274–80.
[127] Machens H-G, Pabst A, Dreyer M, Gliemroth J, Görg S, Bahlmann L, et al. C3a levels and occurrence of subdermal vascular thrombosis are age-related in deep second-degree burn wounds. Surgery 2006;139(4):550–5.
[128] Meevassana J, Serirodom S, Prabsattru P, Boonsongserm P, Kamolratanakul S, Siritientong T, et al. Alu repetitive sequence cpg methylation changes in burn scars. Burns 2022;48(6):1417–24.
[129] Thongsroy J, Patchsung M, Mutirangura A. The association between alu hypomethylation and severity of type 2 diabetes mellitus. Clin. Epigenetics 2017:993.
[130] Patchsung M, Settayanon S, Pongpanich M, Mutirangura D, Jintarith P, Mutirangura A. Alu sirna to increase alu element methylation and prevent DNA damage. Epigenomics 2018;10(2):175–85.
[131] Yasom S, Khumsri W, Boonsongserm P, Kitkumthorn N, Ruangvejvorachai P, Sooksamran A, et al. B1 sirna increases de novo DNA methylation of b1 elements and promotes wound healing in diabetic rats. Front Cell. Dev Biol 2021:9802024.
[132] Sogabe Y, Seno H, Yamamoto T, Yamada Y. Unveiling epigenetic regulation in cancer, aging, and rejuvenation with in vivo reprogramming technology. Cancer Sci 2018;109(9):2641–50.
[133] Pastar I, Marjanovic J, Liang L, Stone RC, Kashpur O, Jozic I, et al. Cellular reprogramming of diabetic foot ulcer fibroblasts triggers pro-healing mirna-mediated epigenetic signature. Exp Dermatol 2021;30(8):1065–72.
[134] Mahmoudi S, Mancini E, Xu L, Moore A, Jahanbani F, Hebestreit K, et al. Heterogeneity in old fibroblasts is linked to variability in reprogramming and wound healing. Nature 2019;574(7779):553–8.
[135] Kashpur O, Smith A, Gerami-Naini B, Maione AG, Calabrese R, Tellechea A, et al. Differentiation of diabetic foot ulcer-derived induced pluripotent stem cells reveals distinct cellular and tissue phenotypes. FASEB J 2019;33 (1):1262–77.
[136] Gill D, Parry A, Santos F, Okkenhaug H, Todd CD, Hernando-Herraez I, et al. Multi-omic rejuvenation of human cells by maturation phase transient reprogramming. Elife 2022;11:e71624.
[137] Kurita M, Araoka T, Hishida T, O'Keefe DD, Takahashi Y, Sakamoto A, et al. In vivo reprogramming of wound-resident cells generates skin epithelial tissue. Nature 2018;561(7722):243–7.
[138] Kaushik K, Das A. Twist1-reprogrammed endothelial cell transplantation potentiates neovascularization-mediated diabetic wound tissue regeneration. Diabetes 2020;69(6):1232–47.
[139] Kim H, Wang SY, Kwak G, Yang Y, Kwon IC, Kim SH. Exosome-guided phenotypic switch of m1 to m2 macrophages for cutaneous wound healing. Adv Sci (Weinh) 2019;6(20):1900513.
[140] Gan J, Liu C, Li H, Wang S, Wang Z, Kang Z, et al. Accelerated wound healing in diabetes by reprogramming the macrophages with particle-induced clustering of the mannose receptors. Biomaterials 2019;219119340.
[141] Basu A, Tiwari VK. Epigenetic reprogramming of cell identity: Lessons from development for regenerative medicine. Clin Epigenetics 2021;13(1):144.
[142] Kim K, Doi A, Wen B, Ng K, Zhao R, Cahan P, et al. Epigenetic memory in induced pluripotent stem cells. Nature 2010;467(7313):285-90.
[143] Katz LS, Geras-Raaka E, Gershengorn MC. Reprogramming adult human dermal fibroblasts to islet-like cells by epigenetic modification coupled to transcription factor modulation. Stem Cells Dev 2013;22(18):2551–60.
[144] Wang W, Yan Y, Guo Z, Hou H, Garcia M, Tan X, et al. All around suboptimal health - a joint position paper of the suboptimal health study consortium and european association for predictive, preventive and personalised medicine. EPMA J 2021;12(4):403–33.
[145] Tachalov VV, Orekhova LY, Kudryavtseva TV, Loboda ES, Pachkoriia MG, Berezkina IV, et al. Making a complex dental care tailored to the person: Population health in focus of predictive, preventive and personalised (3p) medical approach. EPMA J 2021;12(2):129–40.
[146] Brunmair J, Bileck A, Schmidl D, Hagn G, Meier-Menches SM, Hommer N, et al. Metabolic phenotyping of tear fluid as a prognostic tool for personalised medicine exemplified by t2dm patients. EPMA J 2022;13(1):107–23.
[147] Xu T, Yu D, Zhou W, Yu L. A nomogram model for the risk prediction of type 2 diabetes in healthy eastern china residents: A 14-year retrospective cohort study from 15,166 participants. EPMA J 2022;13(3):397–405.

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