



## Review Article

# Hypoxia-inducible Factor-1 $\alpha$ in Diabetic Foot Ulcers: Plain but Not Simple



Xinmeng Zhou<sup>1,2#</sup>, Daojiang Yu<sup>2#</sup>, Xiaodong Sun<sup>1,3</sup>, Wei Huang<sup>3,4</sup>, Yong Xu<sup>3,4</sup>, Changlong Li<sup>2\*</sup>   
and Yuanyuan Zhang<sup>1,5,6,7\*</sup> 

<sup>1</sup>Department of Pharmacology, West China School of Basic Medical Sciences and Forensic Medicine, West China School of Pharmacy, Sichuan University, Chengdu, China; <sup>2</sup>Department of Biochemistry and Molecular Biology, West China School of Basic Medical Sciences and Forensic Medicine, Sichuan University, Chengdu, China; <sup>3</sup>Metabolic Vascular Disease Key Laboratory of Sichuan Province, Luzhou, Sichuan, China; <sup>4</sup>Department of Endocrinology, Affiliated Hospital of Southwest Medical University, Luzhou, China; <sup>5</sup>Key Laboratory of Drug-Targeting and Drug Delivery System of the Education Ministry and Sichuan Province, Sichuan Research Center for Drug Precision Industrial Technology, West China School of Pharmacy, Sichuan University, Chengdu, 610041, China; <sup>6</sup>Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA, USA; <sup>7</sup>Penn Image-Guided Interventions Lab, University of Pennsylvania, Philadelphia, PA, USA

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## Abstract

Hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ ) is usually regarded as a core regulator of hypoxic response. Persistent inflammation and impaired wound healing are common manifestations of diabetic foot ulcer (DFU). In normal wounds, HIF-1 $\alpha$  and its related regulatory molecules, such as vascular endothelial growth factor and inducible nitric oxide synthase, are activated by hypoxia signals, which in turn promote wound healing. However, abnormal regulation of the HIF-1 $\alpha$  signaling pathway by hyperglycemia leads to impaired wound healing in DFU. In this review, we highlight the tissue-specific and stage-specific effects of the HIF-1 $\alpha$  signaling pathway in DFU. In the early stage of DFU, HIF-1 $\alpha$  in inflammatory cells is over-upregulated by hyperglycemia, causing the activation of nuclear factor- $\kappa$ B and the inducible nitric oxide synthase-mediated pro-inflammatory signaling pathway, leading to sustained inflammation, which is deleterious. In the late stage of DFU, HIF-1 $\alpha$  in endothelial cells and keratinocytes is inhibited by hyperglycemia, which leads to the downregulation of vascular endothelial growth factor ex-

pression, resulting in insufficient angiogenesis and difficult healing at the wound site. In this review, we discuss recent advances in the knowledge of the HIF-1 $\alpha$  signaling pathway and the key targeted molecules in impaired wound healing of DFU. We also summarize the drugs currently in clinical trials that target HIF-1 $\alpha$  or its downstream molecules, recapitulate current gaps in our knowledge, and propose rational therapeutic strategies for DFU based on the action characteristics of HIF-1 $\alpha$ .

**Keywords:** Diabetic foot ulcer; Hypoxia-inducible factor-1 $\alpha$ ; Nuclear factor- $\kappa$ B; Inducible nitric oxide synthase; Vascular endothelial growth factor.

**Abbreviations:** AGE, advanced glycation end product; Bax, BCL-2-associated X protein; CBP, CREB-binding protein; CHIP, carboxy terminus of Hsp70-interacting protein; CKD, Chronic Kidney Disease; CXCR4, CXC chemokine receptor 4; DFO, deferoxamine; DFU, diabetic foot ulcer; DMOG, dimethylxallyl glycine; eNOS, endothelial NOS; FIH-1, factor inhibiting HIF-1; HIF-1, hypoxia-inducible factor-1; HIF-1 $\alpha$ , hypoxia-inducible factor-1 alpha; HRE, hypoxia response element; Hsp40, heat shock protein 40; Hsp70, heat shock protein 70; IFN- $\gamma$ , interferon-gamma; I $\kappa$ B, inhibitor of NF- $\kappa$ B; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; MGO, methylglyoxal; NCT, National Clinical Trial; NF- $\kappa$ B, nuclear factor-kappa B; NO, nitric oxide; NOS, NO synthase; PHD, prolyl hydroxylase domain; RAGE, receptor for AGEs; ROS, reactive oxygen species; TBK1, tank-bound kinase 1; TNF- $\alpha$ , tumor necrosis factor alpha; Ub, ubiquitin; VEGF, vascular endothelial growth factor; VEGFR, VEGF receptor; VHL, von Hippel-Lindau; 2-OG, 2-oxyglutaric acid.

\*Correspondence to: Changlong Li, Department of Biochemistry and Molecular Biology, West China School of Basic Medical Sciences and Forensic Medicine, Sichuan University, No. 17 People's South Road, Chengdu 610041, China. ORCID: <https://orcid.org/0009-0009-0699-0731>. Tel: 86-28-85501243, Fax: 86-28-85501243, E-mail: changlongli@scu.edu.cn; Yuanyuan Zhang, Department of Pharmacology, West China School of Basic Medical Sciences and Forensic Medicine, West China School of Pharmacy, Sichuan University, No. 17 People's South Road, Chengdu 610041, China. ORCID: <https://orcid.org/0000-0002-9263-6262>. Tel: 86-28-85501278, Fax: 86-28-85501278, E-mail: zhangyy@scu.edu.cn

#These authors contributed equally to this work.

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## Introduction

Diabetes mellitus is one of the most common chronic diseases in the world, whose incidence keeps growing over the past few decades, making it a major public health challenge.<sup>1,2</sup> According to the International Diabetes Federation, 537 million adults suffered from diabetes worldwide in 2021 and the numbers may rise to 643 million by 2030 and 783 million by 2045.<sup>3</sup> Notably, China was the country with the largest number of adults with diabetes in 2019, estimated as 116 million, and this ranking is not expected to change by 2045.<sup>4</sup> With over 100 complications, diabetes is the disease with the most complications. The most frequent diabetes complications

are well-described and include cardiovascular disease, diabetic nephropathy, diabetic neuropathy, and diabetic foot ulcer (DFU). Emerging complications include various cancers, functional and cognitive impairments, liver disease, and sleep disorders.<sup>5</sup>

As one of the common complications of diabetes, DFU is also a common clinical refractory skin injury mainly caused by peripheral vascular neuropathy.<sup>6,7</sup> In diabetes, the lifetime incidence of DFU is estimated to be 15–25%.<sup>8</sup> According to the data of the International Diabetes Federation in 2015, there are 9.1–26.1 million diabetes patients with foot ulcers worldwide every year.<sup>9</sup> DFU has a 50–70% recurrence rate within 5 years.<sup>10</sup> In China, the recurrence rate in cured DFU patients within 1 year was as high as 31.6%.<sup>11</sup> The prognosis of DFU is poor, with a risk of amputation, cardiovascular and cerebrovascular events, and premature death, which also results in costs that may be even higher than for many common cancers.<sup>12,13</sup> The direct annual cost of DFU in the United States is estimated to be 17 billion US dollars, which is higher than those of common cancers such as breast cancer (16.5 billion) and lung cancer (12.12 billion).<sup>14</sup> Moreover, DFU is the most important risk factor for lower limb amputation, which occurs 10 times more frequently in people with type 2 diabetes than in those without.<sup>15</sup> DFU is responsible for 80% of nontraumatic amputations, of which 85% are due to foot ulcers.<sup>16</sup> Moreover, patients with diabetes and amputations have a higher risk of all-cause death than those who have diabetes but no amputations.<sup>17</sup> A recent meta-analysis showed that the 5-year mortality rate of DFU patients was 49.1% and the 10-year survival rate was only 23.1%.<sup>18</sup>

Currently, the current commonly used therapeutic methods for DFU mainly include antibiotic therapy,<sup>19</sup> local decompression therapy,<sup>20</sup> revascularization surgery,<sup>21</sup> hyperbaric oxygen therapy or ozone therapy,<sup>22</sup> new excipients,<sup>23</sup> and negative pressure therapy.<sup>24</sup> Although antibiotic therapy is the standard treatment for DFU, it cannot reduce amputation risk and mortality since most DFU wounds are infected with highly resistant bacteria.<sup>25</sup> Local decompression therapy such as full contact plaster support restricts patients' activities and induces easy wound infection after long-term wearing.<sup>26</sup> Revascularization surgery, including intravascular revascularization and open revascularization, has a high limb salvage rate but a high postoperative mortality rate, which rises sharply to 13% at 1 year, 29% at 2 years, and 47% at 5 years.<sup>27</sup> The limited mechanical strength and high degradation rate of excipients such as topical gels limit their clinical application.<sup>23</sup> The use of negative pressure therapy is also restricted due to its high cost and limited adaptability.<sup>28</sup> All of the above therapies have low specificity, therefore, targeted therapy is in urgent need to enhance efficacy for abundant DFU patients in the precision medicine era.

Hypoxia-inducible factor-1 alpha (HIF-1 $\alpha$ ) is a potent oxygen-dependent transcriptional factor that regulates the expression of downstream erythropoietic genes and angiogenic-related genes such as vascular endothelial growth factor (VEGF) to adapt to low oxygen stress.<sup>29</sup> Since HIF-1 $\alpha$  is famous for promoting angiogenesis, it is commonly regarded as a beneficial factor in hypoxic disorders such as DFU. HIF-1 $\alpha$  plays complex roles both by itself and by interacting with other molecules in DFU, whose roles are plain but not simple.<sup>30</sup> In this review, we summarize the roles and signaling pathways of HIF-1 $\alpha$  in DFU, and propose potential targeted medications, aiming to provide a new perspective on understanding its mechanism and propose novel strategies for the development of targeted therapies.

### HIF-1 $\alpha$ and its canonical regulatory pathways

In 1991, Semenza and colleagues identified four or more different

nuclear factors by analyzing DNase I hypersensitive sites in hepatocytes.<sup>31</sup> They found that at least two of them were induced under anemic or anoxic conditions. They then discovered a protein complex that binds to a particular piece of DNA and changes accordingly with oxygen concentration, which was named the hypoxia-inducible factor-1 (HIF-1). Since then, HIF-1 has been found to play an important role in hypoxic-ischemia diseases, metabolic diseases,<sup>32</sup> and cancer.<sup>33</sup>

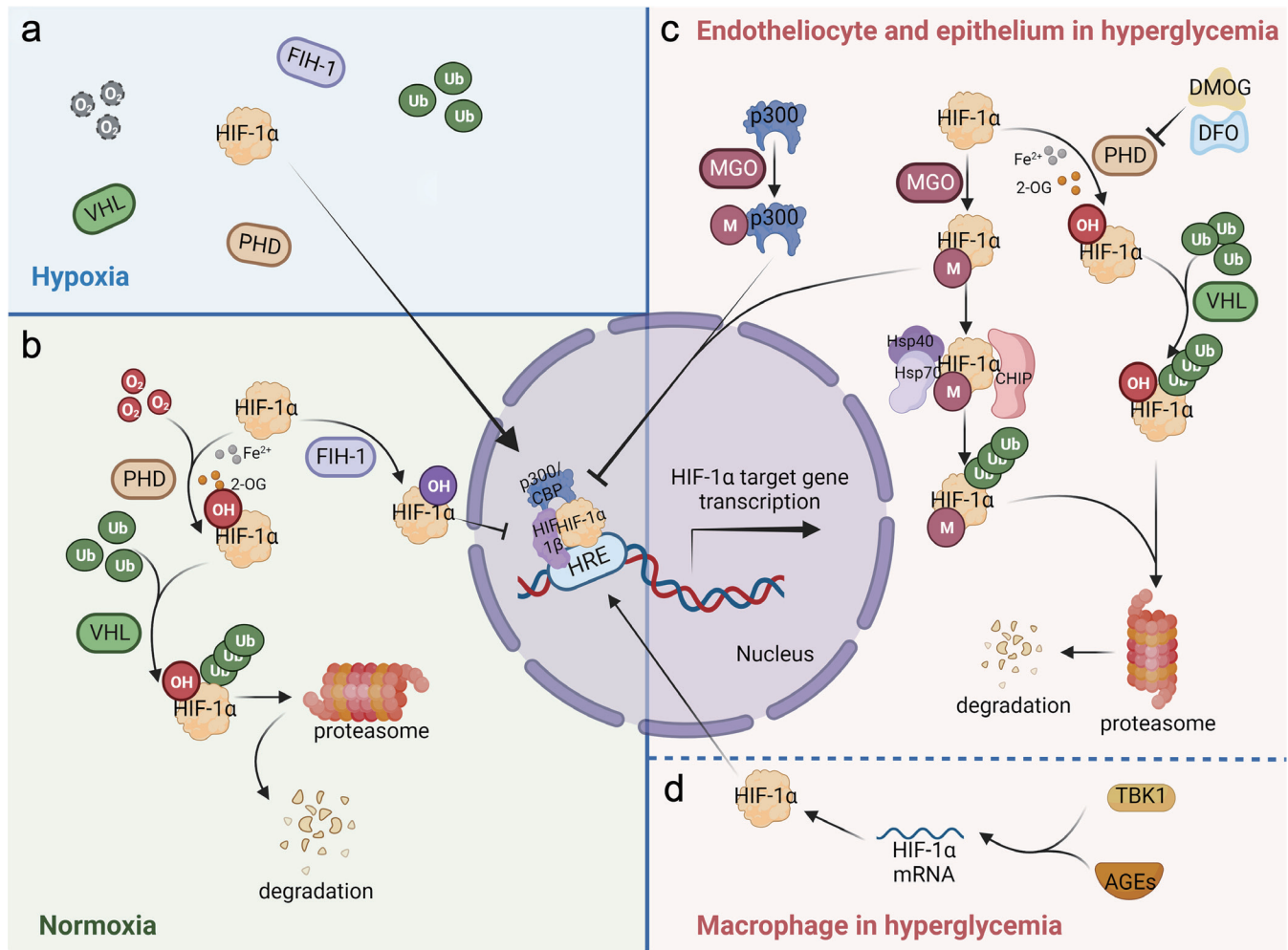
HIF-1 is a heterodimer consisting of an oxygen-regulated 120 kDa  $\alpha$  subunit, HIF-1 $\alpha$ , and a constitutively expressed 91–94 kDa beta subunit whose activity is not affected by oxygen or HIF-1 $\beta$ .<sup>34</sup> Both subunits contain one basic helix-loop-helix domain and two PER-ARNT-SIM domains (A and B), which are required for the dimerization of alpha and beta subunits and the core sequences binding to target-gene promoters.<sup>35</sup> HIF-1 stability and transcriptional activity are mediated via the modulation of HIF-1 $\alpha$  by oxygen, which can activate the transcription of multiple target genes, such as VEGF,<sup>36</sup> inducible nitric oxide synthase (iNOS),<sup>37</sup> and erythropoietin.<sup>38,39</sup>

As a master regulator of cellular oxygen levels, HIF-1 $\alpha$  mediates the cellular adaptive response to hypoxia because of its ability to regulate the expression of many target genes, such as those involved in angiogenesis, cell survival, proliferation, and migration.<sup>40,41</sup> The regulation of HIF-1 $\alpha$  is mainly at the post-translational level, such as hydroxylation,<sup>42</sup> ubiquitination,<sup>43</sup> and methylation.<sup>44</sup> HIF-1 $\alpha$  is hydroxylated by prolyl hydroxylase domain (PHD) proteins at two specific proline residues, P402 and P564, in the presence of normal oxygen, 2-oxyglutaric acid (2-OG), and iron concentrations (Fig. 1b).<sup>45</sup> Hydroxylation of HIF-1 $\alpha$  stimulates its recognition and ubiquitination by the von Hippel–Lindau (VHL) syndrome, an E3 ubiquitin ligase, followed by proteasome degradation.<sup>46</sup> In addition to stability regulation, the trans-activating activity of HIF-1 $\alpha$  can also be mediated by oxygen.<sup>47</sup> Factor inhibiting HIF-1 (FIH-1) hydroxylates HIF-1 $\alpha$  on a specific aspartic acid residue (N803) located in C-terminal transactivation domains under normoxic conditions, prevents the recruitment of coactivators CREB-binding protein (CBP)/p300, and inhibits the transactivation activity of HIF-1 $\alpha$  (Fig. 1b).<sup>48</sup> Under hypoxic conditions, the ability of PHD to hydroxylate HIF-1 $\alpha$  is decreased, leading to the failure of VHL to recognize HIF-1 $\alpha$  and mediate the ubiquitination proteasome degradation pathway, resulting in the enhanced stability of HIF-1 $\alpha$ .<sup>49,50</sup> HIF-1 $\alpha$  translocates to the nucleus where it dimerizes with HIF-1 $\beta$ , binds to the hypoxia response element (HRE) sequence of targets gene promoter, and recruits its coactivators CBP/p300, thereby transactivating the transcription of downstream genes.<sup>51</sup> The activity of FIH-1 is also decreased under hypoxia owing to its use of oxygen as a substrate. Then the recruitment of HIF-1 $\alpha$  coactivators is enhanced, which improves its transactivation activity (Fig. 1a).<sup>42</sup>

### Mechanisms of HIF-1 $\alpha$ in DFU

#### *HIF-1 $\alpha$ is activated at the wound site and plays a positive role in multiple stages of wound healing*

The oxygen supply has an essential role in wound healing.<sup>52</sup> Accordingly, hyperbaric oxygen therapy is widely used to treat ischemic DFU.<sup>53</sup> During tissue injury and remodeling, wounds become oxygen starved and progressively worse due to the rupture of blood vessels and the succeeding increase in oxygen consumption as infiltrated inflammatory cells at the injury site.<sup>54</sup> Subsequently, the stability of HIF-1 $\alpha$  is increased due to hypoxia which facilitates



**Fig. 1. The HIF-1 $\alpha$  signaling under normoxia, hypoxia, and hyperglycemia.** (a) Under hypoxia, HIF-1 $\alpha$  is protected from degradation due to the reduced hydroxylation activity of PHD, translocates to the nucleus to dimerize with HIF-1 $\beta$ , collects the co-activator CBP/p300 in the HRE region, thus activating the transcription of downstream genes. (b) Under normoxia, HIF-1 $\alpha$  is hydroxylated by PHD in the presence of oxygen, 2-OG, and iron, which stimulates the recognition and ubiquitination of HIF-1 $\alpha$  by VHL and succeeding proteasome degradation. The hydroxylation of HIF-1 $\alpha$  by FIH-1 under normoxia prevents the recruitment of coactivators to HIF-1 $\alpha$  to bind to the HRE sequence, resulting in the hindrance of HIF-1 $\alpha$  transactivation activity. (c) In endotheliocyte and epithelium exposed to hyperglycemia, apart from the PHD-VHL-mediated proteasome degradation pathway, high glucose induces the methylation of HIF-1 $\alpha$  and increases its interaction with Hsp40/70 by triggering the accumulation of MGO, which promotes CHIP-mediated polyubiquitination and proteasome degradation. Moreover, MGO-mediated methylation prevents the nuclear translocation of HIF-1 $\alpha$  and the recruitment of the co-activator p300, which decreases the activity of HIF-1 $\alpha$ . DMOG and DFO enhance the stability of HIF-1 $\alpha$  in hyperglycemia by inhibiting the hydroxylation of PHD. (d) Conversely, in macrophages, hyperglycemia promotes the transcription of HIF-1 $\alpha$  via TBK1 and AGEs. 2-OG, 2-oxoglutarate; AGEs, advanced glycation end products; CBP, CREB-binding protein; CHIP, carboxy terminus of Hsp70-interacting protein; DFO, deferoxamine; DMOG, dimethylxalylglycine; FIH-1, factor inhibiting HIF-1; HIF-1 $\alpha$ , hypoxia-inducible factor-1 alpha; HIF-1 $\beta$ , hypoxia-inducible factor-1 beta; HRE, hypoxia response element; Hsp40, heat shock protein 40; Hsp70, heat shock protein 70; MGO, methylglyoxal; PHD, prolyl hydroxylase domain protein; TBK1, tank-bound kinase 1; Ub, ubiquitin; VHL, von Hippel-Lindau protein. Created with BioRender.com.

wound healing through transactivation of the expression of genes involved in angiogenesis, such as angiogenic growth factor.<sup>55</sup>

Previous studies have identified phase-specific and spatiotemporal properties of HIF-1 $\alpha$  and its target genes by measuring their mRNA content in skin cells. Increased HIF-1 $\alpha$  expression was detected in basal keratinocytes at the edge of a wound immediately after injury, was almost undetectable after epithelialization, peaked during re-epithelialization, and was detectable in the dermis at no time during wound healing.<sup>56</sup> This suggests that HIF-1 $\alpha$  has a pleiotropic role in the process of wound healing to ensure that the injured tissues return to normal health homeostasis.

HIF-1 $\alpha$  has benefits in different processes of wound healing.<sup>57</sup> It promotes the transition of cellular mitochondrial oxidative respiration to glycolysis by regulating enzymes such as PDH kinase 1 and decreases the production of reactive oxygen species (ROS) to prevent subsequent tissue damage.<sup>58</sup> In the early stages of wound healing, HIF-1 $\alpha$  promotes migration and infiltration of macrophages and neutrophils by regulating the glycolysis in myeloid cells and increases their killing effect on bacteria to prevent serious infection in the damaged skin barrier.<sup>59</sup> In addition, HIF-1 $\alpha$  promotes angiogenesis under hypoxia by upregulating the transcription of multiple genes, such as VEGF, angiopoietin 2, and transforming growth

factor beta 3, which provide oxygen and nutrients needed for tissue repair.<sup>60,61</sup> HIF-1 $\alpha$  induces the migration of CXC chemokine receptor 4 (CXCR4)-expressing bone marrow-derived circulating endothelial progenitor cells to the hypoxic site by increasing the expression of stromal cell-derived factor-1, also known as CXCL12.<sup>62</sup> Local treatment with AMD3100, a specific CXCR4 antagonist, promoted wound healing in diabetic mice by mobilizing bone marrow endothelial progenitor cells and enhancing both angiogenesis and vasculogenesis.<sup>63</sup> Mice with keratinocyte-specific knockout of HIF-1 $\alpha$  have delayed wound healing and inhibition of keratinocyte migration because of a decrease in laminin-332 and  $\beta$ 1 integrin.<sup>64</sup> Recent studies have shown that overexpression of HIF-1 $\alpha$  can promote the proliferation and migration of vascular endothelial cells under hypoxia.<sup>65</sup> Overall, HIF-1 $\alpha$  is activated by hypoxia at the injury site and promotes wound healing.

#### ***Abnormal regulation of HIF-1 $\alpha$ stability and activity by hyperglycemia has a key role in wound healing deficits in DFU***

The disruption of HIF-1 $\alpha$  and its involved signaling pathways is the key to impaired wound healing in DFU, which is caused by the over-activation or inhibition of HIF-1 $\alpha$  by hyperglycemia in different tissues and cells (Fig. 1c and d). Hyperglycemia results in damaged hypoxia signaling, decreased neovascularization, and subsequent poor wound healing.<sup>66</sup> If cells fail to adapt to hyperglycemia-induced hypoxia, tissue hypoxia is further exacerbated by regulating the stability and transactivation of HIF-1 $\alpha$ .<sup>30</sup> Low levels of HIF-1 $\alpha$  in macrovascular and microvascular diseases are closely associated with hyperglycemia, and restoration of HIF-1 $\alpha$  expression can significantly improve related diseases such as DFU.<sup>57</sup> The protein levels and nuclear translocation of HIF-1 $\alpha$  are more reduced in DFU than in venous ulcers, which have a similar hypoxia profile to DFU but without hyperglycemia.<sup>67</sup> Interestingly, the transcription of HIF-1 $\alpha$  does not change, suggesting that hyperglycemia has multiple regulation mechanisms on HIF-1 $\alpha$ , including impairing HIF-1 $\alpha$ 's hypoxic-dependent protection against proteasome degradation and declining transactivation efficacy of HIF-1 $\alpha$  mediated by blocking its binding to HRE.<sup>68</sup>

Hyperglycemia decreases the stability of HIF-1 $\alpha$  in a PHD- and VHL-dependent manner. Increased HIF-1 $\alpha$  stability accelerates wound healing in diabetic mice due to the blocking of the interaction between VHL and HIF-1 $\alpha$ .<sup>69</sup> Additionally, inhibiting the PHD-dependent degradation of HIF-1 $\alpha$  with hydroxylase inhibitors dimethylxallyl glycine (DMOG) or deferoxamine (DFO) enhances the stability of HIF-1 $\alpha$  in hyperglycemia, which in turn promotes diabetic wound healing (Fig. 1c).<sup>30</sup> These results indicate that the negative regulation of hyperglycemia on HIF-1 $\alpha$  stability is at least partially dependent on the proteasome degradation mediated by PHD and VHL, which is also the cause of delayed healing of diabetic wounds.

Hyperglycemia also promotes HIF-1 $\alpha$  instability independent of PHD and VHL.<sup>70</sup> High glucose induces the accumulation of methylglyoxal (MGO), which further modifies HIF-1 $\alpha$  to increase its interaction with Hsp40/70. Then, the E3 ubiquitin ligase carboxy terminus of Hsp70-interacting protein (CHIP) mediates polyubiquitination and proteasome degradation of HIF-1 $\alpha$  (Fig. 1c).<sup>71</sup> Modification of HIF-1 $\alpha$  and p300 by MGO inhibits the transcriptional activity of HIF-1 $\alpha$  by inhibiting the dimerization of HIF-1, binding to HRE, and interacting with p300 (Fig. 1c).<sup>72,73</sup> As ROS have a key role in hyperglycemia-induced MGO accumulation, inhibition of iron-catalyzed ROS production by DFO can reduce the covalent modification of p300 by MGO, promote the binding of p300 to HIF-1 $\alpha$ , and enhance the transcriptional activity of HIF-

1 $\alpha$ , thus improving DFU.<sup>74</sup>

Although numerous studies have demonstrated that hyperglycemia negatively regulates HIF-1 $\alpha$ , paradoxically, hyperglycemia has also been shown to promote HIF-1 $\alpha$  expression through tank-binding kinase 1 in macrophages and thus promote inflammation.<sup>75</sup> Moreover, the accumulation of advanced glycation end products (AGEs) during hyperglycemia promotes an M1-like proinflammatory polarization of macrophages through upregulating the expression and translocation of HIF-1 $\alpha$ , leading to atherosclerosis, which is also one of the important characteristics of DFU (Fig. 1d).<sup>76,77</sup> This may be explained by the distinct roles of various cells in different stages of DFU. Abnormal regulation of the HIF-1 $\alpha$  signaling pathway has a crucial role in the pathogenesis of DFU. Elucidating the interaction of HIF-1 $\alpha$  with downstream molecules in DFU may provide novel therapeutic targets or biomarkers for DFU.

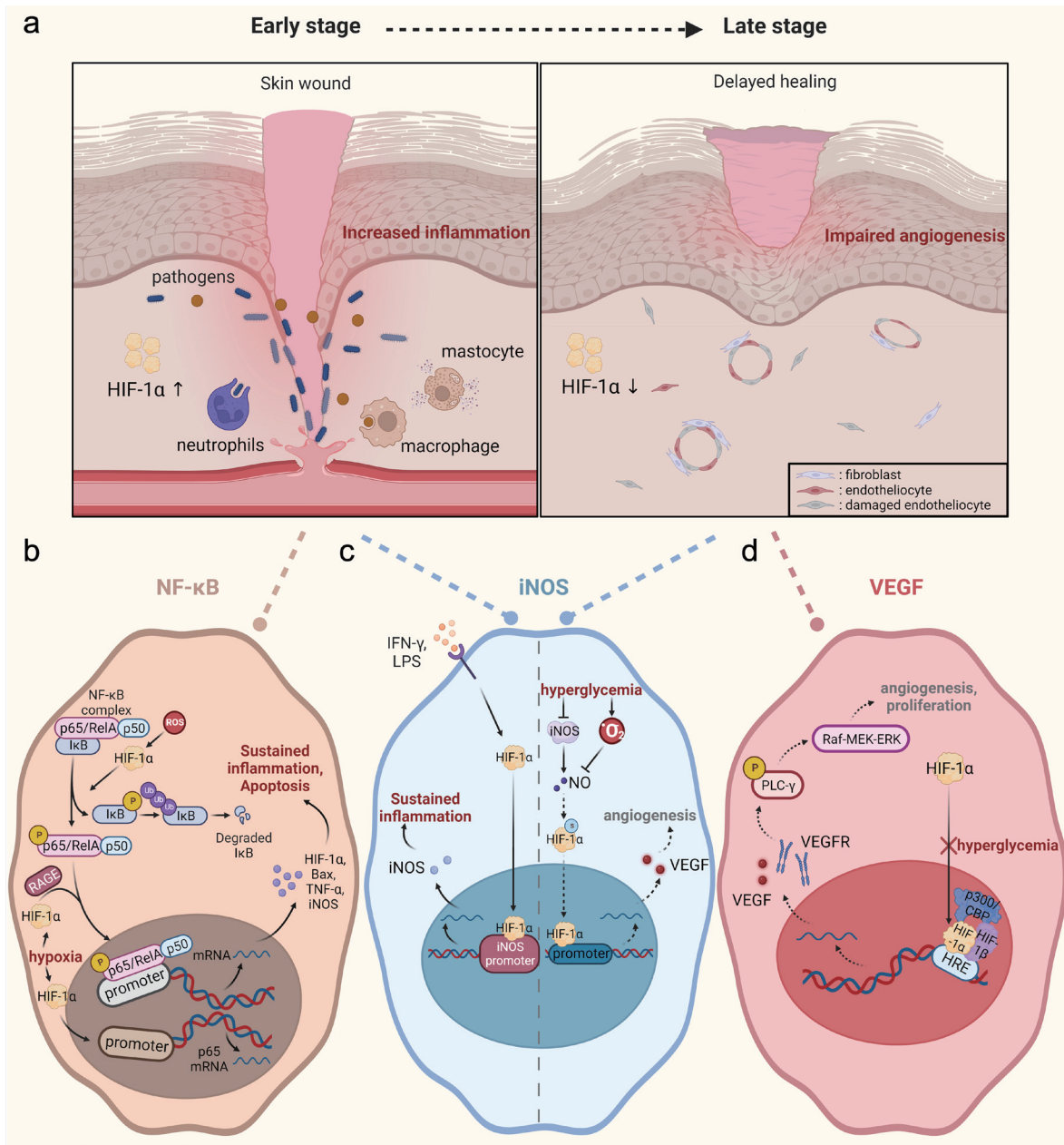
#### ***Impaired HIF-1 $\alpha$ signaling pathway and related molecular dysregulation result in delayed wound healing in DFU***

Impairment of the HIF-1 $\alpha$  signaling pathway and subsequent dysregulation of downstream target proteins result in delayed wound healing in DFU (Fig. 2a). Currently, it is believed that the main characteristics of delayed wound healing in DFU are as follows: In the early stage of wound healing, the over activation of inflammatory cells and the release of pro-inflammatory cytokines lead to persistent low-grade inflammation. When wound healing enters the late stage, impaired granulation tissue formation due to insufficient angiogenesis leads to decreased wound re-epithelialization.<sup>78</sup>

As an oxygen-dependent transcription factor, HIF-1 $\alpha$  activates the transcription of hundreds of downstream genes by binding to the HRE of gene promoters, many of which are involved in inflammation, angiogenesis, and endothelial function.<sup>79</sup> In particular, nuclear factor-kappa B (NF- $\kappa$ B), iNOS/NOS2, and VEGF play a pivotal role in DFU (Fig. 2b-d). NF- $\kappa$ B and its inhibitor I $\kappa$ B constitute a strictly controlled system, which regulates inflammation and the redox status of vascular endothelial cells.<sup>80</sup> Animal studies have shown that inhibition of NF- $\kappa$ B signaling alleviates endothelium-dependent vasodilation function damage.<sup>81,82</sup> It is further clinically confirmed that NF- $\kappa$ B has a key role in endothelial dysfunction.<sup>83</sup> In skin injury, iNOS is the main source of nitric oxide (NO) in damaged endothelial tissues, and it influences wound healing by improving endothelial vasodilation and regulating fibroblast proliferation and collagen synthesis.<sup>84</sup> VEGF is an endothelial cell-specific mitogen promoter, which increases the production of plasminogen activator, degrades extracellular matrix during capillary germination, and enhances vascular permeability before the formation of new blood vessels.<sup>85</sup>

#### ***NF- $\kappa$ B***

NF- $\kappa$ B is a key transcriptional regulator of innate immunity, inflammation, and apoptosis.<sup>86,87</sup> Its downstream signaling pathways have a key role in diabetes.<sup>88</sup> NF- $\kappa$ B was first described by Sen and Baltimore in 1986 as a transcription factor regulating the expression of target genes.<sup>89</sup> The NF- $\kappa$ B family consists of five homologous or heterodimer proteins, p65 (RelA, NF- $\kappa$ B3), c-Rel, RelB, p50/p105 (NF- $\kappa$ B1), and p52/p100 (NF- $\kappa$ B2).<sup>90</sup> RelA, c-Rel, and RelB contain the transcription activation domain rather than p52 and p50, so the latter two need to combine with other factors to have a positive regulatory role in transcription.<sup>86</sup> Among them, the heterodimer NF- $\kappa$ B composed of p65 and p50 is the most abundant and active form.<sup>91</sup> In the nonstimulated resting state, I $\kappa$ B, the inhibitor of NF- $\kappa$ B, binds to NF- $\kappa$ B heterodimers, allowing NF- $\kappa$ B to exist in the cytoplasm and preventing its binding



**Fig. 2. HIF-1 $\alpha$  and its targeted key molecules in DFU.** (a) The role of HIF-1 $\alpha$  in the pathogenesis of DFU is tissue-specific and stage-specific. At the early stage of wound healing in DFU, the expression of HIF-1 $\alpha$  is increased in the injured site, especially in macrophages, causing sustained inflammation. In contrast, the stability and transcriptional activity of HIF-1 $\alpha$  in endothelial cells is decreased by hyperglycemia in the late stage, leading to insufficient angiogenesis and impaired wound healing. (b) There is a mutually reinforcing relationship between HIF-1 $\alpha$  and NF- $\kappa$ B in the hypoxic inflammation of DFU wounds. On one hand, ROS promotes the HIF-1 $\alpha$ -mediated phosphorylation of I $\kappa$ B and p65, which enhances the nuclear translocation of NF- $\kappa$ B and the expression of its target inflammatory cytokines genes. Under hypoxia, HIF-1 $\alpha$  directly upregulates p65 mRNA levels and indirectly promotes the transcriptional activation of NF- $\kappa$ B on downstream genes by upregulating RAGE expression. On the other hand, NF- $\kappa$ B, as a transcription regulator, binds to the HIF-1 $\alpha$  promoter and upregulates the expression of HIF-1 $\alpha$ , which in turn enhances the function of NF- $\kappa$ B. (c) Th1 cytokines, such as IFN- $\gamma$  and LPS, promote the expression of HIF-1 $\alpha$  and act on the iNOS promoter homologous with the HRE sequence, up-regulate the expression of iNOS, which is responsible for the continuous amplification of inflammation. The difference is that during the proliferation phase, decreased iNOS activity and increased superoxide production in diabetic skin decreased the positive regulatory effect of NO on the HIF-1 $\alpha$ /VEGF pathway. (d) Because of defective HIF-1 $\alpha$  signaling in endothelial cells under hyperglycemia, HIF-1 $\alpha$  cannot enter the nucleus and bind to the HRE sequence to promote downstream VEGF and VEGFR expression. Consequently, PLC- $\gamma$  phosphorylation is decreased and the activation of the Raf/MEK/ERK pathway is blocked, which ultimately leads to insufficient angiogenesis and delayed wound healing. Bax, BCL-2-associated X protein; CBP, CREB-binding protein; DFU, diabetic foot ulcer; HIF-1 $\alpha$ , hypoxia-inducible factor-1 alpha; HIF-1 $\beta$ , hypoxia-inducible factor-1 beta; HRE, hypoxia response element; I $\kappa$ B, inhibitor of NF- $\kappa$ B; IFN- $\gamma$ , interferon-gamma; iNOS, inducible nitric oxide synthase; LPS, lipopolysaccharide; NF- $\kappa$ B, nuclear factor-kappa B; NO, nitric oxide; RAGE, receptor for AGEs; ROS, reactive oxygen species; TNF- $\alpha$ , tumor necrosis factor alpha; Ub, ubiquitin; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor. Created with BioRender.com.

to DNA.<sup>92</sup> When stimulated by a pathogen or pro-inflammatory cytokines such as tumor necrosis factor- $\alpha$  and interleukin-1, I $\kappa$ B kinase (IKK) is activated and phosphorylates serine residues of I $\kappa$ B, leading to ubiquitination proteasome degradation.<sup>93,94</sup> Then, NF- $\kappa$ B translocates into the nucleus and binds to the  $\kappa$ B element on DNA to regulate the expression of downstream inflammatory genes, such as iNOS, IL-2, and IL-6.<sup>95,96</sup>

DFU does not follow the normal four stages of wound healing, hemostasis, inflammation, proliferation, and remodeling. DFU manifests as prolonged inflammation that leads to a chronic wound.<sup>97</sup> Sustained inflammation at the wound site is mainly due to the increased production of inflammation-related enzymes and cytokines and the abundant infiltration of inflammatory cells.<sup>98</sup> In addition, fibrosis and thrombosis caused by inflammation lead to obstructed blood and reduced oxygen supply, which also aggravate hypoxia.<sup>99</sup> Therefore, HIF-1 $\alpha$  and NF- $\kappa$ B signaling pathways, as transcriptional regulators of hypoxic adaptive response and inflammatory response, are not independent, but crosstalk and interact with each other (Fig. 2b).<sup>87</sup>

Specifically, NF- $\kappa$ B directly activates and binds to the nuclear factor-activated B cell  $\kappa$  light chain enhancer site on the promoter of HIF-1 $\alpha$ , thereby upregulating the transcription of HIF-1 $\alpha$ .<sup>100</sup> Moreover, HIF-1 $\alpha$ -mediated phosphorylation of I $\kappa$ B and p65 enhances the translocation to the nucleus and transcriptional activity of p65, thereby upregulating the activity of NF- $\kappa$ B.<sup>101</sup> However, hypoxia cannot up-regulate p65 mRNA levels and phosphorylated p65 levels in HIF-1 $\alpha$ -deficient macrophages, as it does in wild-type macrophages.<sup>102</sup> HIF-1 $\alpha$  also indirectly promotes nuclear translocations of NF- $\kappa$ B and the activation of pro-inflammatory genes by upregulating the expression of alarmin receptors, such as the receptor for AGEs (RAGE) (Fig. 2b).<sup>103</sup> An important feature of wound hypoxia in DFU is excess ROS, which plays an important role in regulating the activity and interaction of HIF-1 $\alpha$  and NF- $\kappa$ B. A recent study showed that hyperglycemia increased ROS to enhance the NF- $\kappa$ B-mediated inflammatory response by activating HIF-1 $\alpha$ , resulting in vascular endothelial cell dysfunction in rat aorta.<sup>104</sup> The increased level of human circadian locomotor output cycle protein kaput under hypoxia induces the production of ROS with subsequent activation of the RhoA and NF- $\kappa$ B pathways, which ultimately leads to oxidative damage and inflammation of blood vessels.<sup>105</sup> In chronic diabetic wounds, p-STAT3 is continuously activated after injury, while I $\kappa$ B $\alpha$  lacks a significant upregulation signal during repair, suggesting that continuous activation of NF- $\kappa$ B in diabetic chronic wounds causes excessive inflammation and delayed wound healing.<sup>106</sup> Apart from that, a recent study has shown that JAK1, 3/STAT3 promotes the demethylation of H3K27me3 by Jumonji domain-containing protein D3. Then, the production of NF- $\kappa$ B-mediated inflammatory cytokines is promoted, which leads to persistent inflammation and poor healing in diabetes.<sup>107</sup>

Consequently, over activation of HIF-1 $\alpha$  in the early stage of wound healing is deleterious to DFU wound closure. To be specific, hyperglycemia and hypoxia cause a mutually reinforcing positive feedback loop between HIF-1 $\alpha$  and NF- $\kappa$ B in the early stage of DFU, leading to persistent inflammation, impeding the transition of wound healing from the inflammatory phase to the proliferative phase, and ultimately exhibiting poor healing.

### iNOS

In 1980, Furchgott *et al.* found that the diastolic effect of acetylcholine on blood vessels depended on the integrity of vascular endothelial cells and substances released by them, which he

named endothelium-derived relaxing factor.<sup>108</sup> Subsequently, endothelium-derived relaxing factor was experimentally proved to be NO in 1987.<sup>109</sup> NO is synthesized *in vivo* by an isoenzyme, NO synthase (NOS), which has three subtypes, neuronal NOS, iNOS, and endothelial NOS (eNOS).<sup>110</sup> Neuronal NOS and eNOS are expressed constitutively and iNOS expression is induced only when cells are stimulated.<sup>111</sup> Under normal physiological conditions, NO is mainly produced by eNOS in endothelial cells and has a role in regulating vascular tone and angiogenesis, inhibiting inflammation, and reducing pathological thrombosis.<sup>112</sup> However, when cells are exposed to inflammatory cytokines or bacterial lipopolysaccharides, iNOS is activated to produce abundant NO to defend against infection or inflammation.<sup>113</sup> In addition, iNOS is also involved in VEGF-A-mediated angiogenesis in the early stage of wound healing, promoting the proliferation and angiogenesis of endothelial cells, thus accelerating wound closure.<sup>114,115</sup> However, under pathological conditions, excessive NO produced by the over activation of iNOS leads to the occurrence and development of a variety of complex multifactorial diseases, such as Alzheimer's disease, atherosclerosis, and inflammatory bowel disease.<sup>116</sup> Hyperglycemia-induced iNOS overexpression results in endothelial damage and apoptosis of vascular endothelial cells, and inhibition of iNOS can protect endothelial cells from high glucose-induced injury.<sup>117</sup> Diabetic peripheral neuropathy is one of the most common causes of DFU.<sup>118</sup> Knockdown of iNOS can protect mice from diabetic peripheral neuropathy compared with wild-type littermates.<sup>119</sup>

During normal wound repair, the L-arginine metabolic pathway is spatiotemporally specific and promotes wound healing by mediating inflammation and maintaining normal macrophage function.<sup>120</sup> Systematically speaking, iNOS produced by wound central macrophages is rapidly elevated within hours after trauma, metabolizing L-arginine to NO, thereby mediating the removal of pathogens. Two days after trauma, fibroblasts near the wound produced arginase, which promotes the conversion of L-arginine to ornithine and facilitates collagen deposition, cell growth, and wound healing.<sup>121,122</sup> In the proliferative stage, keratinocytes are the main source of VEGF which is the most important angiogenic factor at this stage. NO, as a kind of ROS, promotes angiogenesis by stabilizing HIF-1 $\alpha$  in endothelial cells and increasing the production of VEGF, thus accelerating diabetic wound healing.<sup>123,124</sup> The stabilizing effect of NO on HIF-1 $\alpha$  may be due to the fact that NO mediates the S-nitrosation of the cysteine residue of HIF-1 $\alpha$  and protects HIF-1 $\alpha$  from degradation by the ubiquitination proteasome pathway.<sup>125</sup>

Notably, decreased NOS activity and increased superoxide production in diabetic wounds as found to reduce the bioavailability of NO and delayed wound healing (Fig. 2c).<sup>126</sup> Therefore, NOS and superoxide dismutase gene therapy during the early stage of wound healing has potential therapeutic effects on chronic wound healing such as DFU.<sup>127</sup> On the contrary, diabetic wounds exhibit persistent inflammation characterized by the inability of M1 macrophages producing iNOS to convert into M2 macrophages producing arginase 1, which leads to the obstruction of the transition from the inflammatory stage to the proliferative stage and impaired healing progress of diabetic wounds.<sup>128</sup> Failure of macrophages to transform from M1 to M2 on the third day of wound formation is a key factor leading to a sustained inflammation of diabetic wounds and impaired wound closure.<sup>129</sup> Therefore, identifying the factors driving macrophage polarization from M1 to M2 is of great importance for the treatment of chronic refractory wounds such as DFU.<sup>130</sup> It has been observed that lipopolysaccharide (LPS) and

**Table 1. Pharmacological agents targeting HIF-1 $\alpha$  in clinical trials**

Drug target	Drug name	Classification of drug action	Indication	Clinical trial status (NCT number)	Reference
HIF-1 $\alpha$	Daprodustat	Agonist	DFU	Phase 1 NCT01831804	162
HIF-1 $\alpha$	Molidustat	Agonist	Anemia associated with CKD	Phase 2 NCT01975818 NCT02021370NCT02021409	161
HIF-1 $\alpha$	Roxadustat	Agonist	CKD anemia in stable dialysis	Phase 3 NCT02273726	161
HIF-1 $\alpha$	EZN-2208	Inhibitor	Refractory solid tumor	Phase 1 NCT01251926	163
HIF-1 $\alpha$	NLG207	Inhibitor	Advanced prostate cancer	Phase 2 NCT02769962	164

CKD, Chronic Kidney Disease; DFU, diabetic foot ulcer; HIF-1 $\alpha$ , hypoxia-inducible factor-1 alpha; NCT, National Clinical Trial.

Th1 cytokines such as interferon- $\gamma$  (IFN- $\gamma$ ) induced M1-type polarization of macrophages and increased the production of iNOS by upregulating the expression of HIF-1 $\alpha$  (Fig. 2c).<sup>131</sup> Similarly, owing to the homology of the iNOS promoter sequence to the HRE sequence, the transcription of the iNOS gene is regulated by HIF-1 $\alpha$ .<sup>132</sup> Under hypoxia, the inflammatory factor IL-1 $\beta$  enhances the transcription of iNOS by promoting HIF-1 to bind to the iNOS promoter.<sup>133</sup> Interestingly, LPS induces activation of IKK and degradation of I $\kappa$ B through toll-like receptors on macrophages, followed by migration of NF- $\kappa$ B into the nucleus and binding to iNOS promoter, triggering the expression of iNOS and inflammation.<sup>113</sup>

Hence, the HIF-1 $\alpha$ /iNOS signaling pathway in DFU is detrimental in the inflammatory stage and beneficial in the proliferative stage. In the inflammatory stage of wound healing, the high expression of HIF-1 $\alpha$  in macrophages inhibits phenotype polarization, resulting in the over-production of iNOS and persistent inflammation. In the proliferative stage, hyperglycemia undermines the activity of iNOS and HIF-1 $\alpha$  in endothelial cells and keratinocytes, resulting in poor angiogenesis and slow wound closure.

### VEGF

VEGF is a specific mitogen family of vascular endothelial cells, which can increase vascular permeability,<sup>134,135</sup> and promote endothelial cells proliferation and migration,<sup>136</sup> angiogenesis,<sup>137</sup> vasculogenesis, collagen deposition, *etc.* Family members include VEGF-A, VEGF-B, VEGF-C, VEGF-D, VEGF-E, and placental growth factor.<sup>138</sup> VEGF-A, commonly referred to as VEGF, includes several isomers, VEGF121, VEGF121b, VEGF145, VEGF145b, VEGF165, VEGF165b, *etc.*<sup>139</sup> In particular, the angiogenic and anti-angiogenic activity of different isomers contribute to endothelial homeostasis.<sup>140</sup> VEGF is a key molecule in tissue repair and wound healing by increasing tissue perfusion by improving vascular permeability and angiogenesis. VEGF is synthesized and secreted by many cells involved in wound healing, such as platelets,<sup>141</sup> macrophages,<sup>142</sup> neutrophils,<sup>143</sup> endothelial cells,<sup>144</sup> fibroblasts,<sup>145</sup> and smooth muscle cells. Local application with the mesoglycan/VEGF at the lesions of mice activated endothelial cells and fibroblasts, accelerated angiogenesis at the wound site, and thus promoted wound repair.<sup>146</sup> CKD712 promotes wound healing by enhancing the migration of fibroblasts through upregulating VEGF, which is weakened by VEGF antagonism.<sup>147</sup> Consistent with this, the wounds of rats treated with topical quercetin showed an increased closure rate, enhanced fibroblast proliferation, raised microvascular density, improved re-epithelialization structure, and more regular collagen deposition compared with controls. These effects are due in part to quercetin's upregulation

of VEGF at the wounds.<sup>148</sup>

HIF-1 $\alpha$  is the main determinant of angiogenesis by activating the transcription of VEGF, which encodes a key angiogenic growth factor.<sup>149</sup> In hypoxic wound tissue, HIF-1 $\alpha$  is stabilized, enters the nucleus, dimerizes with HIF-1 $\beta$ , binds to the promoter HRE of VEGF, and simultaneously recruits the coactivators p300/CBP, triggering the transcription of VEGF and thus playing an essential role in promoting normal wound healing (Fig. 2d).<sup>36,150</sup> Through mutual recognition and binding with VEGF receptors (VEGFR), VEGF exerts the tyrosine-protein kinase activity of VEGFR and phosphorylates various proteins in endothelial cells.<sup>151</sup> For example, VEGF binds to VEGFR to phosphorylate PLC- $\gamma$  and induces subsequent activation of the Raf/MEK/ERK pathway, which then promotes endothelial cell proliferation.<sup>152</sup> Similarly, VEGFR phosphorylates and activates the PI3K/Akt pathway, promotes endothelial cell survival, and improves vascular permeability.<sup>153,154</sup> Moreover, VEGF protects endothelial cells from apoptosis by inducing the expression of anti-apoptotic proteins Bcl-2 and A1.<sup>155</sup> Furthermore, HIF-1 $\alpha$  increases the expression of VEGFR, which is similar to the mechanism of regulating VEGF, thus further enhancing the function of VEGF (Fig. 2d).<sup>156</sup> VEGF promotes angiogenesis and endothelial cell proliferation and migration, making it a promising target for chronic refractory wounds such as DFU.<sup>157</sup> Therefore, hyperglycemia-induced inhibition of the HIF-1 $\alpha$ /VEGF signaling pathway is an important cause and a potential pharmacological target of DFU (Fig. 2d).

Despite its positive role in promoting chronic wound healing in DFU, overexpression of VEGF is considered a critical event aggravating other diabetic complications. VEGF is a downstream effector of STAT3, a key factor in diabetic retinopathy that mediates increased vascular permeability and pathological angiogenesis, whose activation may worsen diabetic retinopathy.<sup>158</sup> VEGF promotes the development of diabetic nephropathy by affecting blood flow and capillary permeability of glomeruli to increase albuminuria.<sup>159</sup> Consistent with this, VEGF antibodies improved albuminuria and glomerular hypertrophy in diabetic rats, which may be an effective strategy for early diabetic nephropathy.<sup>160</sup>

### Pharmacological agents targeting HIF-1 $\alpha$ and related molecules for treating DFU

As HIF-1 $\alpha$  and its downstream molecules play important roles in DFU, they may have potential as pharmacological targets. We summarize drugs targeting HIF-1 $\alpha$  and the three related molecules mentioned above that are currently in development, and which might be used for DFU or contraindicated for DFU (Tables 1–4).<sup>161–175</sup>

**Table 2. Pharmacological agents targeting NF- $\kappa$ B in clinical trials**

Drug target	Drug name	Classification of drug action	Indication	Clinical trial status (NCT number)	Reference
NF- $\kappa$ B	Iguratimod	Inhibitor	Active rheumatoid arthritis	Phase 4 NCT01554917	165
NF- $\kappa$ B	Quinacrine	Inhibitor	Androgen-independent prostate cancer	Phase 2 NCT00417274	166
NF- $\kappa$ B	Vinpocetine	Inhibitor	Acute ischemic stroke	Phase 3 NCT02878772	167

NCT, National Clinical Trial; NF- $\kappa$ B, nuclear factor-kappa B.

**Table 3. Pharmacological agents targeting iNOS in clinical trials**

Drug target	Drug name	Classification of drug action	Indication	Clinical trial status (NCT number)	Reference
iNOS/NOS2	EGb-761	Inhibitor	Mild cognitive impairment; inflammation; oxidative stress	Phase 4 NCT05594355	168,169
iNOS/NOS2	Ronopterin	Inhibitor	Traumatic brain injury	Phase 3 NCT02794168	168

iNOS, inducible nitric oxide synthase; NCT, National Clinical Trial.

### Drugs targeting HIF-1 $\alpha$ and strategies for the treatment of DFU

HIF-1 $\alpha$  promotes wound healing in DFU by enhancing angiogenesis and enhancing endothelial cell function. Therefore, intramuscular injection of HIF-1 $\alpha$  agonists around wounds at the end of wound healing can improve the damaged angiogenesis and thus achieve the therapeutic effect on DFU. Currently, a phase I clinical trial is being conducted to evaluate the safety, tolerability, and pharmacokinetics of daprodustat, an agonist of HIF-1 $\alpha$ , when used topically in healthy individuals and patients with DFU (Table 1).<sup>166</sup> Other HIF-1 $\alpha$  agonists, such as molidustat and roxadustat, are being evaluated in clinical trials to treat anemia caused by chronic kidney disease.<sup>161</sup> Some HIF-1 $\alpha$  agonists are being validated in rat models of DFU. DMOG was found to stabilize HIF-1 $\alpha$  as a PHD inhibitor.<sup>177</sup> Topical use of mono-axial polycaprolactone nanofibers loaded with DMOG promoted wound healing in Sprague Dawley rats with streptozotocin-induced diabetes.<sup>162</sup> Accordingly, local pharmacological inhibition of PHD is a potential therapeutic strategy for DFU. However, there is still a lack of clinical trials with large sample sizes on the efficacy and safety of DMOG for DFU. In addition, smeared VH298 exosomes loaded with gelatin methacryloyl hydrogel promoted angiogenesis and accelerated wound healing of male db/db mice, in which VH298 stabilized HIF-1 $\alpha$  and activated the HIF-1 $\alpha$ -VEGF pathway by inhibiting the binding between VHL and HIF-1 $\alpha$ .<sup>178</sup> Cyclometalated iridium (III) metal complex 1a can promote angiogenesis and wound heal-

ing in diabetic mice under different administration routes such as intraperitoneal injection and topical application by antagonizing the interaction between VHL and HIF-1 $\alpha$  like VH298.<sup>69</sup> These studies suggest the potential advantages of using gene editing to inhibit VHL and block VHL-HIF-1 $\alpha$  interactions in the treatment of chronic wounds such as DFU.<sup>179</sup> However, it is important to note that experimental murine models of diabetes mellitus do not fully mimic human disease.<sup>180</sup> Therefore, the efficacy and safety of the above-mentioned VHL-HIF-1 $\alpha$  interaction inhibitors for DFU need to be tested in scientifically designed large sample-size clinical trials.

Moreover, most clinical trials are being conducted to demonstrate the therapeutic effect of intravenous infusion of HIF-1 $\alpha$  inhibitors such as EZN-2208 and NLG207 on tumors due to their favorable inhibition of angiogenesis and cell proliferation.<sup>163,164</sup> Given the primary role of HIF-1 $\alpha$  in mediating M1 macrophage polarization and inflammation in the early stage of wound healing, the dermal administration of HIF-1 $\alpha$  inhibitors should be tested for DFU patients with persistent inflammation. Taken together, the evidence supports the use of inhibitors in the early stage of wound healing and agonists in the late stage as a reasonable strategy for HIF-1 $\alpha$ -targeted drugs in DFU therapy.

### Drugs targeting NF- $\kappa$ B and strategies for the treatment of DFU

Chronic inflammation mediated by NF- $\kappa$ B signaling contributes to

**Table 4. Pharmacological agents targeting VEGF in clinical trials**

Drug target	Drug name	Classification of drug action	Indication	Clinical trial status (NCT number)	Reference
VEGF	Neovasculgen	Agonist	Peripheral arterial disease	Phase 4 NCT02369809	170
VEGF	AZD-8601	Agonist	Type II diabetes	Phase 1 NCT02935712	171
VEGF	Bevacizumab	Inhibitor	Metastatic colorectal cancer	Phase 3 NCT00719797	172
VEGF	Brolucizumab-dblb	Inhibitor	Active choroidal neovascularization; age related macular degeneration	Phase 3 NCT02307682 NCT02434328	174
VEGF	Dilpacimab	Inhibitor	Advanced solid tumors	Phase 1 NCT01946074	173
VEGF	Faricimab	Inhibitor	Diabetic macular edema	Phase 3 NCT03622593 NCT03622580	175

NCT, National Clinical Trial; VEGF, vascular endothelial growth factor.



the pathogenesis and progression of multiple metabolic diseases.<sup>181</sup> Therefore, inhibiting NF- $\kappa$ B is recognized as a treatment strategy for concomitant inflammation in a variety of diseases. Igaratimod, an inhibitor of NF- $\kappa$ B, has been demonstrated to be effective for active rheumatoid arthritis in phase IV clinical trials.<sup>165</sup> Quinacrine was found to have therapeutic effects on androgen-independent prostate cancer, which may promote wound healing in DFU based on its inhibition of NF- $\kappa$ B transcriptional activity.<sup>166</sup> A phase III clinical trial evaluated that vinpocetine, as an anti-inflammatory agent, alleviated the clinical consequences of acute ischemic stroke and improved outcomes (Table 2).<sup>167</sup> Recent studies have shown that gavage treatment with vinpocetine improved pain sensitivity and movement disorders in diabetic rats, and inhibited inflammation by reducing IL-6 production.<sup>182,183</sup> These studies indicate the potential application of vinpocetine for early DFU, which needs to be validated by further clinical trials. Given the central role of NF- $\kappa$ B in inflammation, we suggest that NF- $\kappa$ B inhibitors should be used against inflammation in the late stages of wound healing for DFU patients to achieve greater therapeutic effects. Although there is still a lack of clinical trials of NF- $\kappa$ B inhibitors for the treatment of DFU, significant progress has been made in animal models. A recent study reported that dihydromyricetin reduced the production of HIF-1 $\alpha$ , iNOS, NF- $\kappa$ B, and other inflammatory factors induced by hyperglycemia and alleviated tissue damage in the aorta of diabetic rats.<sup>104</sup> In addition, a phase II clinical trial is currently being conducted to evaluate the efficacy of dihydromyricetin in the treatment of type 2 diabetes owing to its upregulation of insulin sensitivity and insulin secretion.<sup>184</sup> All these trials provide important clues and insights for the development of dihydromyricetin as a potential medicine for DFU. By and large, in terms of targeting NF- $\kappa$ B for DFU treatment, we suggest that NF- $\kappa$ B inhibitors should be used at the end-inflammatory stage of wound healing in DFU patients to facilitate wound repair.

#### **Drugs targeting iNOS and strategies for the treatment of DFU**

iNOS agonists should be used in the treatment of DFU patients or patients with ischemic or infectious ulcers at the late stage of wound healing of DFU because they produce NO to promote angiogenesis, vasodilation, and exert antibacterial effects.<sup>185</sup> However, there have been few clinical trials to test iNOS agonists for treating DFU, and it needs further exploration. Nonetheless, topical NO supplementation has been developed to suppress infection in DFU, such as the EDX110 system, which is a topical medical device releasing NO (Table 3).<sup>186</sup> It should be noted that iNOS agonists should not be used in DFU patients who are at an inflammatory phase without infection, which may cause or aggregate inflammation to persist.

iNOS inhibitors are currently being evaluated in clinical trials to treat severe inflammation, including EGb-761 for oxidative stress and inflammation in mild cognitive impairment and roloperin for traumatic brain injury.<sup>168,169</sup> High iNOS activity has previously been shown to cause persistent inflammation and be detrimental to normal wound healing.<sup>121</sup> Therefore, for DFU patients with abnormally prolonged inflammation, selective iNOS inhibitors should be locally used to promote wound healing into the proliferative and remodeling stage as soon as possible. Nevertheless, given the increased risk of infection due to the suppression of inflammation that may be caused by topical iNOS inhibitors, antibiotics should be used in combination. Generally speaking, we recommend that in the early stage of wound healing, iNOS inhibitors combined with antibiotic therapy can improve inflammation of DFU wound healing. Additionally, iNOS agonists should be injected around the

wound tissue of DFU patients in the late stage of wound healing to promote angiogenesis.

#### **Drugs targeting VEGF and strategies for the treatment of DFU**

As one of the most effective angiogenic factors, VEGF is considered to be an important molecule in promoting wound healing by accelerating angiogenesis.<sup>187</sup> Therefore, intramuscular or intravenous administration of VEGF agonists at the wound site may be beneficial for patients with ischemic ulcers or DFU in the advanced stage of wound healing. Neovascugen is currently being investigated in clinical trials as a VEGF agonist for peripheral artery disease.<sup>170</sup> A phase I clinical trial has also been conducted to evaluate the safety, tolerability, and pharmacodynamics of VEGF agonist, AZD8601, in men with type 2 diabetes to provide evidence for its clinical use for DFU (Table 4).<sup>171</sup> It is suggested that VEGF agonists have more obvious advantages for DFU than current therapies. However, further human clinical trials with large sample sizes are needed to assess its efficacy and safety.

Compared with VEGF agonists, VEGF inhibitors have aroused more interest and attention. For example, many clinical trials have been conducted to investigate the effects of selective VEGF inhibitors on tumors, since the formation of new blood vessels is the basis for tumor growth and metastasis.<sup>188</sup> A phase III trial of bevacizumab showed that VEGF inhibitors could be used to treat metastatic colorectal cancer.<sup>172</sup> The safety and efficacy of dilpocimab in patients with advanced solid tumors were also evaluated and had satisfactory efficacy.<sup>173</sup> Furthermore, as a key promoter of intraocular angiogenesis, VEGF inhibitors are also used for ocular diseases such as macular edema and active choroidal angiogenesis.<sup>174,175</sup> Due to severe wound dysangiogenesis in DFU, VEGF inhibitors cannot be used for DFU patients, even if they have tumors that are an indication of VEGF inhibitors. In conclusion, for the treatment of DFU with targeted VEGF, the late stage of wound healing is the appropriate time to use VEGF agonists. In addition, it should be noted that DFU patients are contraindicated for VEGF inhibitors.

#### **Conclusions and perspectives**

The roles of HIF-1 $\alpha$  and its key interacting molecules including NF- $\kappa$ B, iNOS, and VEGF in the pathogenesis of DFU were discussed in detail in this review. It is noteworthy that HIF-1 $\alpha$  is not fully beneficial for DFU wound healing as commonly believed. Specifically, cross-perturbation between HIF-1 $\alpha$  and NF- $\kappa$ B, HIF-1 $\alpha$ , and iNOS causes harmful persistent inflammation. The upregulation of VEGF induced by HIF-1 $\alpha$  plays a beneficial effect on wound healing by promoting angiogenesis, which is also positively regulated by iNOS. However, the results of current experimental evidence on the signaling pathways involved among these molecules do not constitute a complete story. The specific molecular mechanisms of the crosstalk between NF- $\kappa$ B and HIF-1 $\alpha$  signaling pathways remain unclear. Particularly, there are few studies on the interaction between them under hyperglycemia. It is also unclear how HIF-1 $\alpha$ , as a transcription regulator, regulates the phosphorylation of I $\kappa$ B and RelA in diabetes. Th1 cytokines such as IFN- $\gamma$  and LPS mediate inflammation by activating HIF-1 $\alpha$  to upregulate iNOS expression, but how IFN- $\gamma$  and LPS regulate the expression of HIF-1 $\alpha$  has not been elucidated. In addition, LPS can also influence iNOS via NF- $\kappa$ B, which provides a new perspective for deciphering the interaction between HIF-1 $\alpha$ , NF- $\kappa$ B, and iNOS. Artificial intelligence-developed tools can help clarify the interactions between HIF-1 $\alpha$  and downstream molecules, such as

molecular surface interaction fingerprinting.<sup>189</sup> Moreover, single-cell sequencing of different cells in wound tissue, such as macrophages and endothelial cells, is expected to discover previously unknown molecules and potential pharmacological targets in DFU.

Based on the role of HIF-1 $\alpha$  and related key molecules in different stages of wound healing in DFU, targeted therapy strategies are proposed in this review. iNOS agonists may be an effective treatment for severely infected DFU patients in the early stage. For DFU patients who are in the advanced stage of wound healing inflammation or with lingering inflammation, local application of HIF-1 $\alpha$  inhibitor, NF- $\kappa$ B inhibitor, or iNOS inhibitor to the affected area can reduce inflammation and promote wound closure. However, special attention needs to be paid to the increased risk of infection caused by iNOS inhibitors, which may require antibiotics. Intramuscular or intravenous administration of HIF-1 $\alpha$ , iNOS, or VEGF agonists can restore damaged angiogenesis and accelerate wound healing for DFU in the advanced stage of wound healing or with ischemic manifestations.

Current preclinical and clinical trials of DFU-targeted drugs have limitations. Firstly, there are few clinical trials of DFU-targeted drugs, and most have small sample sizes, which is a major bottleneck. Secondly, current clinical trials enroll patients with DFU at different stages and do not perform subgroup analysis by stage. For example, clinical trials that include both DFU patients at the early stage of inflammation and severe infection to evaluate the efficacy of an iNOS inhibitor often do not produce valid data. Therefore, the inclusion criteria should be formulated according to the Wanger grading criteria of DFU and targeted outcomes to ensure high-quality evidence. Finally, clinical medication strategies should be stage-specific for DFU patients, which is currently neglected. A better understanding of the progression of DFU and making good use of various therapeutic methods to accurately identify the current disease stage and specific etiology of the patient will improve the therapeutic effects.

In conclusion, HIF-1 $\alpha$  signaling has tissue- and stage-specific roles in DFU. Specifically, in the early stage of DFU wound healing, hyperglycemia and hypoxia upregulate HIF-1 $\alpha$  in inflammatory cells such as macrophages, causing excessive activation of pro-inflammatory signaling pathways such as NF- $\kappa$ B and iNOS, resulting in sustained local inflammation and impaired wound healing. In the late stage of DFU wound healing, the HIF-1 $\alpha$  signaling in vascular endothelial cells and keratinocytes is inhibited by hyperglycemia, resulting in decreased expression of its downstream molecule VEGF. Thus, angiogenesis is blocked, which ultimately leads to undermined wound closure. Therefore, pharmacological targeting of HIF-1 $\alpha$  and downstream molecules have potential therapeutic effects for DFU, which should be used according to stages. In the early stage of DFU wound healing, inhibitors of HIF-1 $\alpha$ , NF- $\kappa$ B, or iNOS can effectively inhibit excessive inflammatory damage in the wounds. In the late stage, agonists of HIF-1 $\alpha$ , iNOS, or VEGF should be used, possibly locally, to promote the angiogenesis of wounds to increase blood supply and accelerate healing. Additions deciphering HIF-1 $\alpha$  signaling will provide novel strategies and targets for DFU.

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### Conflict of interest

YYZ has been an editorial board member of *Gene Expression* since February 2023. The authors declare no conflict of interests.

### Author contributions

Searched the literature and prepared the manuscript (XMZ, DJY), reviewed the literature and generated the figures (XDS), reviewed the literature and improved the manuscript (WH, YX), conceived, designed, and edited the manuscript and supervised the study (CLL, YYZ). All authors have read and approved the manuscript.

### References

- [1] Cloete L. Diabetes mellitus: an overview of the types, symptoms, complications and management. *Nurs Stand* 2022;37(1):61–66. doi:10.7748/ns.2021.e11709, PMID:34708622.
- [2] Zimmet P, Alberti KG, Shaw J. Global and societal implications of the diabetes epidemic. *Nature* 2001;414(6865):782–787. doi:10.1038/414782a, PMID:11742409.
- [3] Magliano DJ, Boyko EJ, IDF Diabetes Atlas 10th edition scientific committee. IDF DIABETES ATLAS [Internet]. 10th ed. Brussels: International Diabetes Federation; 2021. PMID:35914061.
- [4] Saeedi P, Petersohn I, Salpea P, Malanda B, Karuranga S, Unwin N, *et al*. Global and regional diabetes prevalence estimates for 2019 and projections for 2030 and 2045: Results from the International Diabetes Federation Diabetes Atlas, 9th edition. *Diabetes Res Clin Pract* 2019;157:107843. doi:10.1016/j.diabres.2019.107843, PMID:31518657.
- [5] Tomic D, Shaw JE, Magliano DJ. The burden and risks of emerging complications of diabetes mellitus. *Nat Rev Endocrinol* 2022;18(9):525–539. doi:10.1038/s41574-022-00690-7, PMID:35668219.
- [6] Jeffcoate WJ, Harding KG. Diabetic foot ulcers. *Lancet* 2003;361(9368):1545–1551. doi:10.1016/S0140-6736(03)13169-8, PMID:12737879.
- [7] Nowak NC, Menichella DM, Miller R, Paller AS. Cutaneous innervation in impaired diabetic wound healing. *Transl Res* 2021;236:87–108. doi:10.1016/j.trsl.2021.05.003, PMID:34029747.
- [8] Centers for Disease Control and Prevention (CDC). History of foot ulcer among persons with diabetes—United States, 2000–2002. *MMWR Morb Mortal Wkly Rep* 2003;52(45):1098–1102. PMID:14614407.
- [9] Rahelic D. 7th Edition of Idf Diabetes Atlas—Call for Immediate Action. *Lijec Vjesn* 2016;138(1-2):57–58. PMID:27290816.
- [10] Boulton AJ, Vileikyte L, Ragnarson-Tennvall G, Apelqvist J. The global burden of diabetic foot disease. *Lancet* 2005;366(9498):1719–1724. doi:10.1016/S0140-6736(05)67698-2, PMID:16291066.
- [11] Jiang Y, Wang X, Xia L, Fu X, Xu Z, Ran X, *et al*. A cohort study of diabetic patients and diabetic foot ulceration patients in China. *Wound Repair Regen* 2015;23(2):222–230. doi:10.1111/wrr.12263, PMID:25682850.
- [12] Ghanassia E, Villon L, Thuan Dit Dieudonné JF, Boegner C, Avignon A, Sultan A. Long-term outcome and disability of diabetic patients hospitalized for diabetic foot ulcers: a 6.5-year follow-up study. *Diabetes Care* 2008;31(7):1288–1292. doi:10.2337/dc07-2145, PMID:18390801.
- [13] Morbach S, Furchert H, Gröblichhoff U, Hoffmeier H, Kersten K, Klauke GT, *et al*. Long-term prognosis of diabetic foot patients and their limbs: amputation and death over the course of a decade.

- Diabetes Care 2012;35(10):2021–2027. doi:10.2337/dc12-0200, PMID:22815299.
- [14] Barshes NR, Sigireddi M, Wrobel JS, Mahankali A, Robbins JM, Kougas P, *et al*. The system of care for the diabetic foot: objectives, outcomes, and opportunities. *Diabet Foot Ankle* 2013;4:1. doi:10.3402/dfa.v4i0.21847, PMID:24130936.
- [15] Déruaz-Luyet A, Raabe C, Garry EM, Brodovicz KG, Lavery LA. Incidence of lower extremity amputations among patients with type 1 and type 2 diabetes in the United States from 2010 to 2014. *Diabetes Obes Metab* 2020;22(7):1132–1140. doi:10.1111/dom.14012, PMID:32090430.
- [16] Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. *JAMA* 2005;293(2):217–228. doi:10.1001/jama.293.2.217, PMID:15644549.
- [17] Hoffstad O, Mitra N, Walsh J, Margolis DJ. Diabetes, lower-extremity amputation, and death. *Diabetes Care* 2015;38(10):1852–1857. doi:10.2337/dc15-0536, PMID:26203063.
- [18] Chen L, Sun S, Gao Y, Ran X. Global mortality of diabetic foot ulcer: A systematic review and meta-analysis of observational studies. *Diabetes Obes Metab* 2023;25(1):36–45. doi:10.1111/dom.14840, PMID:36054820.
- [19] Lipsky BA, Armstrong DG, Citron DM, Tice AD, Morgenstern DE, Abramson MA. Ertapenem versus piperacillin/tazobactam for diabetic foot infections (SIDESTEP): prospective, randomised, controlled, double-blinded, multicentre trial. *Lancet* 2005;366(9498):1695–1703. doi:10.1016/S0140-6736(05)67694-5, PMID:16291062.
- [20] Mueller MJ, Diamond JE, Sinacore DR, Delitto A, Blair VP 3rd, Drury DA, *et al*. Total contact casting in treatment of diabetic plantar ulcers. Controlled clinical trial. *Diabetes Care* 1989;12(6):384–388. doi:10.2337/diacare.12.6.384, PMID:2659299.
- [21] Houliand K. Surgical revascularization and reconstruction procedures in diabetic foot ulceration. *Diabetes Metab Res Rev* 2020;36(Suppl 1):e3256. doi:10.1002/dmrr.3256, PMID:31840931.
- [22] Martínez-Sánchez G, Al-Dalain SM, Menéndez S, Re L, Giuliani A, Candelario-Jalil E, *et al*. Therapeutic efficacy of ozone in patients with diabetic foot. *Eur J Pharmacol* 2005;523(1-3):151–161. doi:10.1016/j.ejphar.2005.08.020, PMID:16198334.
- [23] Bardill JR, Laughter MR, Stager M, Liechty KW, Krebs MD, Zgheib C. Topical gel-based biomaterials for the treatment of diabetic foot ulcers. *Acta Biomater* 2022;138:73–91. doi:10.1016/j.actbio.2021.10.045, PMID:34728428.
- [24] Liu Z, Dumville JC, Hinchliffe RJ, Cullum N, Game F, Stubbs N, *et al*. Negative pressure wound therapy for treating foot wounds in people with diabetes mellitus. *Cochrane Database Syst Rev* 2018;10(10):CD010318. doi:10.1002/14651858.CD010318.pub3, PMID:30328611.
- [25] Chang M, Nguyen TT. Strategy for Treatment of Infected Diabetic Foot Ulcers. *Acc Chem Res* 2021;54(5):1080–1093. doi:10.1021/acs.accounts.0c00864, PMID:33596041.
- [26] Messenger G, Masoetsa R, Hussain I. A Narrative Review of the Benefits and Risks of Total Contact Casts in the Management of Diabetic Foot Ulcers. *J Am Coll Clin Wound Spec* 2017;9(1-3):19–23. doi:10.1016/j.jccw.2018.05.002, PMID:30591897.
- [27] Forsythe RO, Apelqvist J, Boyko EJ, Fitridge R, Hong JP, Katsanos K, *et al*. Effectiveness of revascularisation of the ulcerated foot in patients with diabetes and peripheral artery disease: A systematic review. *Diabetes Metab Res Rev* 2020;36(Suppl 1):e3279. doi:10.1002/dmrr.3279, PMID:32176439.
- [28] Rayman G, Vas P, Dhataria K, Driver V, Hartemann A, Londahl M, *et al*. Guidelines on use of interventions to enhance healing of chronic foot ulcers in diabetes (IWGDF 2019 update). *Diabetes Metab Res Rev* 2020;36(Suppl 1):e3283. doi:10.1002/dmrr.3283, PMID:32176450.
- [29] Lee JW, Bae SH, Jeong JW, Kim SH, Kim KW. Hypoxia-inducible factor (HIF-1) $\alpha$ : its protein stability and biological functions. *Exp Mol Med* 2004;36(1):1–12. doi:10.1038/emm.2004.1, PMID:15031665.
- [30] Botusan IR, Sunkari VG, Savu O, Catrina AI, Grünler J, Lindberg S, *et al*. Stabilization of HIF-1 $\alpha$  is critical to improve wound healing in diabetic mice. *Proc Natl Acad Sci U S A* 2008;105(49):19426–19431. doi:10.1073/pnas.0805230105, PMID:19057015.
- [31] Semenza GL, Neffelt MK, Chi SM, Antonarakis SE. Hypoxia-inducible nuclear factors bind to an enhancer element located 3' to the human erythropoietin gene. *Proc Natl Acad Sci U S A* 1991;88(13):5680–5684. doi:10.1073/pnas.88.13.5680, PMID:2062846.
- [32] Dong P, Li Q, Han H. HIF-1 $\alpha$  in cerebral ischemia (Review). *Mol Med Rep* 2022;25(2):41. doi:10.3892/mmr.2021.12557, PMID:34878158.
- [33] Infantino V, Santarsiero A, Convertini P, Todisco S, Iacobazzi V. Cancer Cell Metabolism in Hypoxia: Role of HIF-1 as Key Regulator and Therapeutic Target. *Int J Mol Sci* 2021;22(11):5703. doi:10.3390/ijms22115703, PMID:34071836.
- [34] Li H, Ko HP, Whitlock JP. Induction of phosphoglycerate kinase 1 gene expression by hypoxia. Roles of Arnt and HIF1 $\alpha$ . *J Biol Chem* 1996;271(35):21262–21267. doi:10.1074/jbc.271.35.21262, PMID:8702901.
- [35] Jiang BH, Rue E, Wang GL, Roe R, Semenza GL. Dimerization, DNA binding, and transactivation properties of hypoxia-inducible factor 1. *J Biol Chem* 1996;271(30):17771–17778. doi:10.1074/jbc.271.30.17771, PMID:8663540.
- [36] Forsythe JA, Jiang BH, Iyer NV, Agani F, Leung SW, Koos RD, *et al*. Activation of vascular endothelial growth factor gene transcription by hypoxia-inducible factor 1. *Mol Cell Biol* 1996;16(9):4604–4613. doi:10.1128/MCB.16.9.4604, PMID:8756616.
- [37] Griffiths EA, Pritchard SA, Welch IM, Price PM, West CM. Is the hypoxia-inducible factor pathway important in gastric cancer? *Eur J Cancer* 2005;41(18):2792–2805. doi:10.1016/j.ejca.2005.09.008, PMID:16290133.
- [38] Weidemann A, Johnson RS. Biology of HIF-1 $\alpha$ . *Cell Death Differ* 2008;15(4):621–627. doi:10.1038/cdd.2008.12, PMID:18259201.
- [39] AbdelMassih A, Yacoub E, Husseiny RJ, Kamel A, Hozaien R, El Shershaby M, *et al*. Hypoxia-inducible factor (HIF): The link between obesity and COVID-19. *Obes Med* 2021;22:100317. doi:10.1016/j.obmed.2020.100317, PMID:33521378.
- [40] Catrina SB, Zheng X. Disturbed hypoxic responses as a pathogenic mechanism of diabetic foot ulcers. *Diabetes Metab Res Rev* 2016;32(Suppl 1):179–185. doi:10.1002/dmrr.2742, PMID:26453314.
- [41] Choudhry H, Harris AL. Advances in Hypoxia-Inducible Factor Biology. *Cell Metab* 2018;27(2):281–298. doi:10.1016/j.cmet.2017.10.005, PMID:29129785.
- [42] Lee FS, Percy MJ. The HIF pathway and erythrocytosis. *Annu Rev Pathol* 2011;6:165–192. doi:10.1146/annurev-pathol-011110-130321, PMID:20939709.
- [43] Ivan M, Kondo K, Yang H, Kim W, Valiando J, Ohh M, *et al*. HIF1 $\alpha$  targeted for VHL-mediated destruction by proline hydroxylation: implications for O<sub>2</sub> sensing. *Science* 2001;292(5516):464–468. doi:10.1126/science.1059817, PMID:11292862.
- [44] Bao L, Chen Y, Lai HT, Wu SY, Wang JE, Hatanpaa KJ, *et al*. Methylation of hypoxia-inducible factor (HIF)-1 $\alpha$  by G9a/GLP inhibits HIF-1 transcriptional activity and cell migration. *Nucleic Acids Res* 2018;46(13):6576–6591. doi:10.1093/nar/gky449, PMID:29860315.
- [45] Kaelin WG Jr, Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell* 2008;30(4):393–402. doi:10.1016/j.molcel.2008.04.009, PMID:18498744.
- [46] Tanimoto K, Makino Y, Pereira T, Poellinger L. Mechanism of regulation of the hypoxia-inducible factor-1  $\alpha$  by the von Hippel-Lindau tumor suppressor protein. *EMBO J* 2000;19(16):4298–4309. doi:10.1093/emboj/19.16.4298, PMID:10944113.
- [47] Lendahl U, Lee KL, Yang H, Poellinger L. Generating specificity and diversity in the transcriptional response to hypoxia. *Nat Rev Genet* 2009;10(12):821–832. doi:10.1038/nrg2665, PMID:19884889.
- [48] Lando D, Peet DJ, Whelan DA, Gorman JJ, Whitelaw ML. Asparagine hydroxylation of the HIF transactivation domain a hypoxic switch. *Science* 2002;295(5556):858–861. doi:10.1126/science.1068592, PMID:11823643.
- [49] Nakayama K, Frew IJ, Hagensen M, Skals M, Habelhah H, Bhoumik A, *et al*. Siah2 regulates stability of prolyl-hydroxylases, controls HIF1 $\alpha$  abundance, and modulates physiological responses to hypoxia. *Cell* 2004;117(7):941–952. doi:10.1016/j.cell.2004.06.001, PMID:15210114.
- [50] Choi HJ, Song BJ, Gong YD, Gwak WJ, Soh Y. Rapid degradation of hypoxia-inducible factor-1 $\alpha$  by KRH102053, a new activator of prolyl hydroxylase 2. *Br J Pharmacol* 2008;154(1):114–125. doi:10.1038/bjp.2008.70, PMID:18332861.
- [51] Catrina SB, Zheng X. Hypoxia and hypoxia-inducible factors in dia-

- betes and its complications. *Diabetologia* 2021;64(4):709–716. doi:10.1007/s00125-021-05380-z, PMID:33496820.
- [52] Desmet CM, Pr at V, Gallez B. Nanomedicines and gene therapy for the delivery of growth factors to improve perfusion and oxygenation in wound healing. *Adv Drug Deliv Rev* 2018;129:262–284. doi:10.1016/j.addr.2018.02.001, PMID:29448035.
- [53] Wenhui L, Changgeng F, Lei X, Baozhong Y, Guobin L, Weijing F. Hyperbaric oxygen therapy for chronic diabetic foot ulcers: An overview of systematic reviews. *Diabetes Res Clin Pract* 2021;176:108862. doi:10.1016/j.diabres.2021.108862, PMID:34015392.
- [54] Lokmic Z, Musyoka J, Hewitson TD, Darby IA. Hypoxia and hypoxia signaling in tissue repair and fibrosis. *Int Rev Cell Mol Biol* 2012; 296:139–185. doi:10.1016/B978-0-12-394307-1.00003-5, PMID:225 59939.
- [55] Guo J, Hu Z, Yan F, Lei S, Li T, Li X, *et al*. Angelica dahurica promoted angiogenesis and accelerated wound healing in db/db mice via the HIF-1 $\alpha$ /PDGF- $\beta$  signaling pathway. *Free Radic Biol Med* 2020;160:447–457. doi:10.1016/j.freeradbiomed.2020.08.015, PMID:32853721.
- [56] Elson DA, Ryan HE, Snow JW, Johnson R, Arbeit JM. Coordinate up-regulation of hypoxia inducible factor (HIF)-1 $\alpha$  and HIF-1 target genes during multi-stage epidermal carcinogenesis and wound healing. *Cancer Res* 2000;60(21):6189–6195. PMID:11085544.
- [57] Jim nez-Jim nez C, Lara-Chica M, Palomares B, Collado JA, Lopez-Miranda J, Mu oz E, *et al*. Effect of N-acyl-dopamines on beta cell differentiation and wound healing in diabetic mice. *Biochim Biophys Acta Mol Cell Res* 2018;1865(11 Pt A):1539–1551. doi:10.1016/j.bbamcr.2018.08.008, PMID:30327197.
- [58] Kim JW, Tchernyshyov I, Semenza GL, Dang CV. HIF-1-mediated expression of pyruvate dehydrogenase kinase: a metabolic switch required for cellular adaptation to hypoxia. *Cell Metab* 2006;3(3):177–185. doi:10.1016/j.cmet.2006.02.002, PMID:16517405.
- [59] Cramer T, Yamanishi Y, Clausen BE, F rster I, Pawlinski R, Mackman N, *et al*. HIF-1 $\alpha$  is essential for myeloid cell-mediated inflammation. *Cell* 2003;112(5):645–657. doi:10.1016/S0092-8674(03)00154-5, PMID:12628185.
- [60] Liu Y, Cox SR, Morita T, Kourembanas S. Hypoxia regulates vascular endothelial growth factor gene expression in endothelial cells. Identification of a 5' enhancer. *Circ Res* 1995;77(3):638–643. doi:10.1161/01.res.77.3.638, PMID:7641334.
- [61] Simon MP, Tournaire R, Pouyssegur J. The angiopoietin-2 gene of endothelial cells is up-regulated in hypoxia by a HIF binding site located in its first intron and by the central factors GATA-2 and Ets-1. *J Cell Physiol* 2008;217(3):809–818. doi:10.1002/jcp.21558, PMID:18720385.
- [62] Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME, *et al*. Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med* 2004;10(8):858–864. doi:10.1038/nm1075, PMID:15235597.
- [63] Nishimura Y, Li M, Qin G, Hamada H, Asai J, Takenaka H, *et al*. CXCR4 antagonist AMD3100 accelerates impaired wound healing in diabetic mice. *J Invest Dermatol* 2012;132(3 Pt 1):711–720. doi:10.1038/jid.2011.356, PMID:22048734.
- [64] Rezvani HR, Ali N, Serrano-Sanchez M, Dubus P, Varon C, Ged C, *et al*. Loss of epidermal hypoxia-inducible factor-1 $\alpha$  accelerates epidermal aging and affects re-epithelialization in human and mouse. *J Cell Sci* 2011;124(Pt 24):4172–4183. doi:10.1242/jcs.082370, PMID:22193962.
- [65] Sun J, Shen H, Shao L, Teng X, Chen Y, Liu X, *et al*. HIF-1 $\alpha$  overexpression in mesenchymal stem cell-derived exosomes mediates cardioprotection in myocardial infarction by enhanced angiogenesis. *Stem Cell Res Ther* 2020;11(1):373. doi:10.1186/s13287-020-01881-7, PMID:32859268.
- [66] Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound Healing: A Cellular Perspective. *Physiol Rev* 2019;99(1):665–706. doi:10.1152/physrev.00067.2017, PMID:30475656.
- [67] Clyne CA, Ramsden WH, Chant AD, Webster JH. Oxygen tension on the skin of the gaiter area of limbs with venous disease. *Br J Surg* 1985;72(8):644–647. doi:10.1002/bjs.1800720820, PMID:4027541.
- [68] Catrina SB, Okamoto K, Pereira T, Brismar K, Poellinger L. Hyperglycemia regulates hypoxia-inducible factor-1 $\alpha$  protein stability and function. *Diabetes* 2004;53(12):3226–3232. doi:10.2337/diabetes.53.12.3226, PMID:15561954.
- [69] Li G, Ko CN, Li D, Yang C, Wang W, Yang GJ, *et al*. A small molecule HIF-1 $\alpha$  stabilizer that accelerates diabetic wound healing. *Nat Commun* 2021;12(1):3363. doi:10.1038/s41467-021-23448-7, PMID:340 99651.
- [70] Bento CF, Fernandes R, Ramalho J, Marques C, Shang F, Taylor A, *et al*. The chaperone-dependent ubiquitin ligase CHIP targets HIF-1 $\alpha$  for degradation in the presence of methylglyoxal. *PLoS One* 2010;5(11):e15062. doi:10.1371/journal.pone.0015062, PMID:21124777.
- [71] Xiao H, Gu Z, Wang G, Zhao T. The possible mechanisms underlying the impairment of HIF-1 $\alpha$  pathway signaling in hyperglycemia and the beneficial effects of certain therapies. *Int J Med Sci* 2013;10(10):1412–1421. doi:10.7150/ijms.5630, PMID:23983604.
- [72] Ceradini DJ, Yao D, Grogan RH, Callaghan MJ, Edelstein D, Brownlee M, *et al*. Decreasing intracellular superoxide corrects defective ischemia-induced new vessel formation in diabetic mice. *J Biol Chem* 2008;283(16):10930–10938. doi:10.1074/jbc.M707451200, PMID:18227068.
- [73] Thangarajah H, Yao D, Chang EI, Shi Y, Jazayeri L, Vial IN, *et al*. The molecular basis for impaired hypoxia-induced VEGF expression in diabetic tissues. *Proc Natl Acad Sci U S A* 2009;106(32):13505–13510. doi:10.1073/pnas.0906670106, PMID:19666581.
- [74] Thangarajah H, Vial IN, Grogan RH, Yao D, Shi Y, Januszyn M, *et al*. HIF-1 $\alpha$  pathway dysfunction in diabetes. *Cell Cycle* 2010;9(1):75–79. doi:10.4161/cc.9.1.10371, PMID:20016290.
- [75] Li Q, Liu Y, Xia X, Sun H, Gao J, Ren Q, *et al*. Activation of macrophage TBK1-HIF-1 $\alpha$ -mediated IL-17/IL-10 signaling by hyperglycemia aggravates the complexity of coronary atherosclerosis: An in vivo and in vitro study. *FASEB J* 2021;35(5):e21609. doi:10.1096/fj.202100086RR, PMID:33908659.
- [76] Han X, Ma W, Zhu Y, Sun X, Liu N. Advanced glycation end products enhance macrophage polarization to the M1 phenotype via the HIF-1 $\alpha$ /PDK4 pathway. *Mol Cell Endocrinol* 2020;514:110878. doi:10.1016/j.mce.2020.110878, PMID:32464167.
- [77] Ji J, Zhao X, Huang J, Wu X, Xie F, Li L, *et al*. Apolipoprotein A-IV of diabetic-foot patients upregulates tumor necrosis factor  $\alpha$  expression in microfluidic arterial models. *Exp Biol Med* (Maywood) 2023;248(8):691–701. doi:10.1177/15353702221147562, PMID:36775868.
- [78] Dixon D, Edmonds M. Managing Diabetic Foot Ulcers: Pharmacotherapy for Wound Healing. *Drugs* 2021;81(1):29–56. doi:10.1007/s40265-020-01415-8, PMID:33382445.
- [79] Yang Y, Lu H, Chen C, Lyu Y, Cole RN, Semenza GL. HIF-1 Interacts with TRIM28 and DNA-PK to release paused RNA polymerase II and activate target gene transcription in response to hypoxia. *Nat Commun* 2022;13(1):316. doi:10.1038/s41467-021-27944-8, PMID:35031618.
- [80] Pierce JW, Read MA, Ding H, Lusinskas FW, Collins T. Salicylates inhibit I kappa B-alpha phosphorylation, endothelial-leukocyte adhesion molecule expression, and neutrophil transmigration. *J Immunol* 1996;156(10):3961–3969. PMID:8621937.
- [81] Sheehan M, Wong HR, Hake PW, Malhotra V, O'Connor M, Zingarelli B. Parthenolide, an inhibitor of the nuclear factor-kappaB pathway, ameliorates cardiovascular derangement and outcome in endotoxic shock in rodents. *Mol Pharmacol* 2002;61(5):953–963. doi:10.1124/mol.61.5.953, PMID:11961112.
- [82] Ungvari Z, Orosz Z, Labinskyy N, Rivera A, Xiangmin Z, Smith K, *et al*. Increased mitochondrial H2O2 production promotes endothelial NF-kappaB activation in aged rat arteries. *Am J Physiol Heart Circ Physiol* 2007;293(1):H37–H47. doi:10.1152/ajpheart.01346.2006, PMID:17416599.
- [83] Pierce GL, Lesniewski LA, Lawson BR, Beske SD, Seals DR. Nuclear factor-(kappa)B activation contributes to vascular endothelial dysfunction via oxidative stress in overweight/obese middle-aged and older humans. *Circulation* 2009;119(9):1284–1292. doi:10.1161/CIRCULATIONAHA.108.804294, PMID:19237660.
- [84] Shi HP, Most D, Efron DT, Tantry U, Fischel MH, Barbul A. The role of iNOS in wound healing. *Surgery* 2001;130(2):225–229. doi:10.1067/msy.2001.115837, PMID:11490353.
- [85] Hyder SM, Stancel GM, Chiappetta C, Murthy L, Boettger-Tong HL, Makela S. Uterine expression of vascular endothelial growth factor is increased by estradiol and tamoxifen. *Cancer Res* 1996;56(17):3954–

3960. PMID:8752163.
- [86] Hayden MS, Ghosh S. Shared principles in NF-kappaB signaling. *Cell* 2008;132(3):344–362. doi:10.1016/j.cell.2008.01.020, PMID:18267068.
- [87] Cummins EP, Keogh CE, Crean D, Taylor CT. The role of HIF in immunity and inflammation. *Mol Aspects Med* 2016;47-48:24–34. doi:10.1016/j.mam.2015.12.004, PMID:26768963.
- [88] Morigi M, Angioletti S, Imberti B, Donadelli R, Micheletti G, Figliuzzi M, *et al.* Leukocyte-endothelial interaction is augmented by high glucose concentrations and hyperglycemia in a NF-kB-dependent fashion. *J Clin Invest* 1998;101(9):1905–1915. doi:10.1172/JCI656, PMID:9576755.
- [89] Sen R, Baltimore D. Multiple nuclear factors interact with the immunoglobulin enhancer sequences. *Cell* 1986;46(5):705–716. doi:10.1016/0092-8674(86)90346-6, PMID:3091258.
- [90] Hayden MS, Ghosh S. Signaling to NF-kappaB. *Genes Dev* 2004;18(18):2195–2224. doi:10.1101/gad.1228704, PMID:15371334.
- [91] Huxford T, Huang DB, Malek S, Ghosh G. The crystal structure of the IkappaBalpha/NF-kappaB complex reveals mechanisms of NF-kappaB inactivation. *Cell* 1998;95(6):759–770. doi:10.1016/s0092-8674(00)81699-2, PMID:9865694.
- [92] Ito CY, Kazantsev AG, Baldwin AS Jr. Three NF-kappa B sites in the I kappa B-alpha promoter are required for induction of gene expression by TNF alpha. *Nucleic Acids Res* 1994;22(18):3787–3792. doi:10.1093/nar/22.18.3787, PMID:7937093.
- [93] Zandi E, Chen Y, Karin M. Direct phosphorylation of IkappaB by IKKalpha and IKKbeta: discrimination between free and NF-kappaB-bound substrate. *Science* 1998;281(5381):1360–1363. doi:10.1126/science.281.5381.1360, PMID:9721103.
- [94] Chen Z, Hagler J, Palombella VJ, Melandri F, Scherer D, Ballard D, *et al.* Signal-induced site-specific phosphorylation targets I kappa B alpha to the ubiquitin-proteasome pathway. *Genes Dev* 1995;9(13):1586–1597. doi:10.1101/gad.9.13.1586, PMID:7628694.
- [95] Baldwin AS Jr. The NF-kappa B and I kappa B proteins: new discoveries and insights. *Annu Rev Immunol* 1996;14:649–683. doi:10.1146/annurev.immunol.14.1.649, PMID:8717528.
- [96] Karin M, Ben-Neriah Y. Phosphorylation meets ubiquitination: the control of NF-[kappa]B activity. *Annu Rev Immunol* 2000;18:621–663. doi:10.1146/annurev.immunol.18.1.621, PMID:10837071.
- [97] Huang F, Lu X, Yang Y, Yang Y, Li Y, Kuai L, *et al.* Microenvironment-Based Diabetic Foot Ulcer Nanomedicine. *Adv Sci (Weinh)* 2023;10(2):e2203308. doi:10.1002/adv.202203308, PMID:36424137.
- [98] Campbell EL, Bruyninckx WJ, Kelly CJ, Glover LE, McNamee EN, Bowers BE, *et al.* Transmigrating neutrophils shape the mucosal microenvironment through localized oxygen depletion to influence resolution of inflammation. *Immunity* 2014;40(1):66–77. doi:10.1016/j.immuni.2013.11.020, PMID:24412613.
- [99] Flück K, Fandrey J. Oxygen sensing in intestinal mucosal inflammation. *Pflügers Arch* 2016;468(1):77–84. doi:10.1007/s00424-015-1722-4, PMID:26206140.
- [100] Bonello S, Zähringer C, BelAiba RS, Djordjevic T, Hess J, Michiels C, *et al.* Reactive oxygen species activate the HIF-1alpha promoter via a functional NFkappaB site. *Arterioscler Thromb Vasc Biol* 2007;27(4):755–761. doi:10.1161/01.ATV.0000258979.92828.bc, PMID:17272744.
- [101] Scortegagna M, Cataisson C, Martin RJ, Hicklin DJ, Schreiber RD, Yuspa SH, *et al.* HIF-1alpha regulates epithelial inflammation by cell autonomous NFkappaB activation and paracrine stromal remodeling. *Blood* 2008;111(7):3343–3354. doi:10.1182/blood-2007-10-115758, PMID:18199827.
- [102] Fang HY, Hughes R, Murdoch C, Coffelt SB, Biswas SK, Harris AL, *et al.* Hypoxia-inducible factors 1 and 2 are important transcriptional effectors in primary macrophages experiencing hypoxia. *Blood* 2009;114(4):844–859. doi:10.1182/blood-2008-12-195941, PMID:19454749.
- [103] Tafani M, Russo A, Di Vito M, Sale P, Pellegrini L, Schito L, *et al.* Up-regulation of pro-inflammatory genes as adaptation to hypoxia in MCF-7 cells and in human mammary invasive carcinoma microenvironment. *Cancer Sci* 2010;101(4):1014–1023. doi:10.1111/j.1349-7006.2010.01493.x, PMID:20151982.
- [104] Awad EM, Ahmed AF, El-Daly M, Amin AH, El-Tahawy NFG, Wagdy A, *et al.* Dihydropyridin protects against high glucose-induced endothelial dysfunction: Role of HIF-1 $\alpha$ /ROR2/NF-kB. *Biomed Pharmacother* 2022;153:113308. doi:10.1016/j.biopha.2022.113308, PMID:35752009.
- [105] Tang X, Guo D, Lin C, Shi Z, Qian R, Fu W, *et al.* hCLOCK Causes Rho-Kinase-Mediated Endothelial Dysfunction and NF-kB-Mediated Inflammatory Responses. *Oxid Med Cell Longev* 2015;2015:671839. doi:10.1155/2015/671839, PMID:26583060.
- [106] Seitz O, Schürmann C, Hermes N, Müller E, Pfeilschifter J, Frank S, *et al.* Wound healing in mice with high-fat diet- or ob gene-induced diabetes-obesity syndromes: a comparative study. *Exp Diabetes Res* 2010;2010:476969. doi:10.1155/2010/476969, PMID:21318183.
- [107] Audu CO, Melvin WJ, Joshi AD, Wolf SJ, Moon JY, Davis FM, *et al.* Macrophage-specific inhibition of the histone demethylase JMJD3 decreases STING and pathologic inflammation in diabetic wound repair. *Cell Mol Immunol* 2022;19(11):1251–1262. doi:10.1038/s41423-022-00919-5, PMID:36127466.
- [108] Furchgott RF, Zawadzki JV. The obligatory role of endothelial cells in the relaxation of arterial smooth muscle by acetylcholine. *Nature* 1980;288(5789):373–376. doi:10.1038/288373a0, PMID:6253831.
- [109] Palmer RM, Ferrige AG, Moncada S. Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 1987;327(6122):524–526. doi:10.1038/327524a0, PMID:3495737.
- [110] Förstermann U, Sessa WC. Nitric oxide synthases: regulation and function. *Eur Heart J* 2012;33(7):829–837. doi:10.1093/eurheartj/ehr304, PMID:21890489.
- [111] Kubes P. Inducible nitric oxide synthase: a little bit of good in all of us. *Gut* 2000;47(1):6–9. doi:10.1136/gut.47.1.6, PMID:10861252.
- [112] Cyr AR, Huckaby LV, Shiva SS, Zuckerbraun BS. Nitric Oxide and Endothelial Dysfunction. *Crit Care Clin* 2020;36(2):307–321. doi:10.1016/j.ccc.2019.12.009, PMID:32172815.
- [113] Cinelli MA, Do HT, Miley GP, Silverman RB. Inducible nitric oxide synthase: Regulation, structure, and inhibition. *Med Res Rev* 2020;40(1):158–189. doi:10.1002/med.21599, PMID:31192483.
- [114] Ziche M, Morbidelli L, Choudhuri R, Zhang HT, Donnini S, Granger HJ, *et al.* Nitric oxide synthase lies downstream from vascular endothelial growth factor-induced but not basic fibroblast growth factor-induced angiogenesis. *J Clin Invest* 1997;99(11):2625–2634. doi:10.1172/JCI119451, PMID:9169492.
- [115] Chin LC, Kumar P, Palmer JA, Rophael JA, Dolderer JH, Thomas GP, *et al.* The influence of nitric oxide synthase 2 on cutaneous wound angiogenesis. *Br J Dermatol* 2011;165(6):1223–1235. doi:10.1111/j.1365-2133.2011.10599.x, PMID:21895624.
- [116] Minhas R, Bansal Y, Bansal G. Inducible nitric oxide synthase inhibitors: A comprehensive update. *Med Res Rev* 2020;40(3):823–855. doi:10.1002/med.21636, PMID:31502681.
- [117] Qi J, Wu Q, Cheng Q, Chen X, Zhu M, Miao C. High Glucose Induces Endothelial COX2 and iNOS Expression via Inhibition of Monomethyltransferase SETD8 Expression. *J Diabetes Res* 2020;2020:2308520. doi:10.1155/2020/2308520, PMID:32185234.
- [118] Liu J, Yuan X, Liu J, Yuan G, Sun Y, Zhang D, *et al.* Risk Factors for Diabetic Peripheral Neuropathy, Peripheral Artery Disease, and Foot Deformity Among the Population With Diabetes in Beijing, China: A Multicenter, Cross-Sectional Study. *Front Endocrinol (Lausanne)* 2022;13:824215. doi:10.3389/fendo.2022.824215, PMID:35733764.
- [119] Vareniuk I, Pavlov IA, Obrosova IG. Inducible nitric oxide synthase gene deficiency counteracts multiple manifestations of peripheral neuropathy in a streptozotocin-induced mouse model of diabetes. *Diabetologia* 2008;51(11):2126–2133. doi:10.1007/s00125-008-1136-3, PMID:18802679.
- [120] Albina JE, Mills CD, Henry WL Jr, Caldwell MD. Temporal expression of different pathways of L-arginine metabolism in healing wounds. *J Immunol* 1990;144(10):3877–3880. PMID:2322635.
- [121] Jude EB, Boulton AJ, Ferguson MW, Appleton I. The role of nitric oxide synthase isoforms and arginase in the pathogenesis of diabetic foot ulcers: possible modulatory effects by transforming growth factor beta 1. *Diabetologia* 1999;42(6):748–757. doi:10.1007/s001250051224, PMID:10382596.
- [122] Abd El-Aleem SA, Abdelwahab S, Am-Sherief H, Sayed A. Cellular and physiological upregulation of inducible nitric oxide synthase, ar-

- ginase, and inducible cyclooxygenase in wound healing. *J Cell Physiol* 2019;234(12):23618–23632. doi:10.1002/jcp.28930, PMID:31161614.
- [123] Yamamoto N, Oyaizu T, Enomoto M, Horie M, Yuasa M, Okawa A, *et al*. VEGF and bFGF induction by nitric oxide is associated with hyperbaric oxygen-induced angiogenesis and muscle regeneration. *Sci Rep* 2020;10(1):2744. doi:10.1038/s41598-020-59615-x, PMID:32066777.
- [124] Yang Y, Huang K, Wang M, Wang Q, Chang H, Liang Y, *et al*. Ubiquitination Flow Repressors: Enhancing Wound Healing of Infectious Diabetic Ulcers through Stabilization of Polyubiquitinated Hypoxia-Inducible Factor-1 $\alpha$  by Theranostic Nitric Oxide Nanogenerators. *Adv Mater* 2021;33(45):e2103593. doi:10.1002/adma.202103593, PMID:34553427.
- [125] Cai Z, Lu Q, Ding Y, Wang Q, Xiao L, Song P, *et al*. Endothelial Nitric Oxide Synthase-Derived Nitric Oxide Prevents Dihydrofolate Reductase Degradation via Promoting S-Nitrosylation. *Arterioscler Thromb Vasc Biol* 2015;35(11):2366–2373. doi:10.1161/ATVBAHA.115.305796, PMID:26381869.
- [126] Hink U, Li H, Mollnau H, Oelze M, Matheis E, Hartmann M, *et al*. Mechanisms underlying endothelial dysfunction in diabetes mellitus. *Circ Res* 2001;88(2):E14–E22. doi:10.1161/01.res.88.2.e14, PMID:11157681.
- [127] Luo JD, Wang YY, Fu WL, Wu J, Chen AF. Gene therapy of endothelial nitric oxide synthase and manganese superoxide dismutase restores delayed wound healing in type 1 diabetic mice. *Circulation* 2004;110(16):2484–2493. doi:10.1161/01.CIR.0000137969.87365.05, PMID:15262829.
- [128] Krzyszczyk P, Schloss R, Palmer A, Berthiaume F. The Role of Macrophages in Acute and Chronic Wound Healing and Interventions to Promote Pro-wound Healing Phenotypes. *Front Physiol* 2018;9:419. doi:10.3389/fphys.2018.00419, PMID:29765329.
- [129] Louiselle AE, Niemiec SM, Zgheib C, Liechty KW. Macrophage polarization and diabetic wound healing. *Transl Res* 2021;236:109–116. doi:10.1016/j.trsl.2021.05.006, PMID:34089902.
- [130] Ganesh GV, Ramkumar KM. Macrophage mediation in normal and diabetic wound healing responses. *Inflamm Res* 2020;69(4):347–363. doi:10.1007/s00011-020-01328-y, PMID:32146517.
- [131] Takeda N, O'Dea EL, Doedens A, Kim JW, Weidemann A, Stockmann C, *et al*. Differential activation and antagonistic function of HIF-1 $\alpha$  isoforms in macrophages are essential for NO homeostasis. *Genes Dev* 2010;24(5):491–501. doi:10.1101/gad.1881410, PMID:20194441.
- [132] Melillo G, Musso T, Sica A, Taylor LS, Cox GW, Varesio L. A hypoxia-responsive element mediates a novel pathway of activation of the inducible nitric oxide synthase promoter. *J Exp Med* 1995;182(6):1683–1693. doi:10.1084/jem.182.6.1683, PMID:7500013.
- [133] Jung F, Palmer LA, Zhou N, Johns RA. Hypoxic regulation of inducible nitric oxide synthase via hypoxia inducible factor-1 in cardiac myocytes. *Circ Res* 2000;86(3):319–325. doi:10.1161/01.res.86.3.319, PMID:10679484.
- [134] Gavard J, Gutkind JS. VEGF controls endothelial-cell permeability by promoting the beta-arrestin-dependent endocytosis of VE-cadherin. *Nat Cell Biol* 2006;8(11):1223–1234. doi:10.1038/ncb1486, PMID:17060906.
- [135] Brkovic A, Sirois MG. Vascular permeability induced by VEGF family members in vivo: role of endogenous PAF and NO synthesis. *J Cell Biochem* 2007;100(3):727–737. doi:10.1002/jcb.21124, PMID:17115409.
- [136] Yamagishi S, Yonekura H, Yamamoto Y, Fujimori H, Sakurai S, Tanaka N, *et al*. Vascular endothelial growth factor acts as a pericyte mitogen under hypoxic conditions. *Lab Invest* 1999;79(4):501–509. PMID:10212003.
- [137] Leung DW, Cachianes G, Kuang WJ, Goeddel DV, Ferrara N. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 1989;246(4935):1306–1309. doi:10.1126/science.2479986, PMID:2479986.
- [138] Rauniyar K, Bokharaie H, Jeltsch M. Expansion and collapse of VEGF diversity in major clades of the animal kingdom. *Angiogenesis* 2023;26(3):437–461. doi:10.1007/s10456-023-09874-9, PMID:37017884.
- [139] Holmes DL, Zachary I. The vascular endothelial growth factor (VEGF) family: angiogenic factors in health and disease. *Genome Biol* 2005;6(2):209. doi:10.1186/gb-2005-6-2-209, PMID:15693956.
- [140] Crafts TD, Jensen AR, Blocher-Smith EC, Markel TA. Vascular endothelial growth factor: therapeutic possibilities and challenges for the treatment of ischemia. *Cytokine* 2015;71(2):385–393. doi:10.1016/j.cyto.2014.08.005, PMID:25240960.
- [141] Banks RE, Forbes MA, Kinsey SE, Stanley A, Ingham E, Walters C, *et al*. Release of the angiogenic cytokine vascular endothelial growth factor (VEGF) from platelets: significance for VEGF measurements and cancer biology. *Br J Cancer* 1998;77(6):956–964. doi:10.1038/bjc.1998.158, PMID:9528841.
- [142] Vannella KM, Wynn TA. Mechanisms of Organ Injury and Repair by Macrophages. *Annu Rev Physiol* 2017;79:593–617. doi:10.1146/annurev-physiol-022516-034356, PMID:27959618.
- [143] Gaudry M, Bregerie O, Andrieu V, El Benna J, Pocard MA, Hakim J. Intracellular pool of vascular endothelial growth factor in human neutrophils. *Blood* 1997;90(10):4153–4161. PMID:9354686.
- [144] Namiki A, Brogi E, Kearney M, Kim EA, Wu T, Couffignal T, *et al*. Hypoxia induces vascular endothelial growth factor in cultured human endothelial cells. *J Biol Chem* 1995;270(52):31189–31195. doi:10.1074/jbc.270.52.31189, PMID:8537383.
- [145] Nissen NN, Polverini PJ, Koch AE, Volin MV, Gamelli RL, DiPietro LA. Vascular endothelial growth factor mediates angiogenic activity during the proliferative phase of wound healing. *Am J Pathol* 1998;152(6):1445–1452. PMID:9626049.
- [146] Belvedere R, Novizio N, Morello S, Petrella A. The combination of mesoglycan and VEGF promotes skin wound repair by enhancing the activation of endothelial cells and fibroblasts and their cross-talk. *Sci Rep* 2022;12(1):11041. doi:10.1038/s41598-022-15227-1, PMID:35773320.
- [147] Jang HJ, Tsoyi K, Kim YM, Park EJ, Park SW, Kim HJ, *et al*. S)-1- $\alpha$ -naphthylmethyl-6,7-dihydroxy-1,2,3,4-tetrahydroisoquinoline (CKD712), promotes wound closure by producing VEGF through HO-1 induction in human dermal fibroblasts and mouse skin. *Br J Pharmacol* 2013;168(6):1485–1496. doi:10.1111/bph.12031, PMID:23088309.
- [148] Gopalakrishnan A, Ram M, Kumawat S, Tandan S, Kumar D. Quercetin accelerated cutaneous wound healing in rats by increasing levels of VEGF and TGF-beta1. *Indian J Exp Biol* 2016;54(3):187–195. PMID:27145632.
- [149] Krock BL, Skuli N, Simon MC. Hypoxia-induced angiogenesis: good and evil. *Genes Cancer* 2011;2(12):1117–1133. doi:10.1177/1947601911423654, PMID:22866203.
- [150] He Y, Munday JS, Perrott M, Wang G, Liu X. Association of Age with the Expression of Hypoxia-Inducible Factors HIF-1 $\alpha$ , HIF-2 $\alpha$ , HIF-3 $\alpha$  and VEGF in Lung and Heart of Tibetan Sheep. *Animals (Basel)* 2019;9(9):673. doi:10.3390/ani9090673, PMID:31514457.
- [151] Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med* 2003;9(6):669–676. doi:10.1038/nm0603-669, PMID:12778165.
- [152] Takahashi T, Ueno H, Shibuya M. VEGF activates protein kinase C-dependent, but Ras-independent Raf-MEK-MAP kinase pathway for DNA synthesis in primary endothelial cells. *Oncogene* 1999;18(13):2221–2230. doi:10.1038/sj.onc.1202527, PMID:10327068.
- [153] Gerber HP, McMurtrey A, Kowalski J, Yan M, Keyt BA, Dixit V, *et al*. Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/Akt signal transduction pathway. Requirement for Flk-1/KDR activation. *J Biol Chem* 1998;273(46):30336–30343. doi:10.1074/jbc.273.46.30336, PMID:9804796.
- [154] Fujio Y, Walsh K. Akt mediates cytoprotection of endothelial cells by vascular endothelial growth factor in an anchorage-dependent manner. *J Biol Chem* 1999;274(23):16349–16354. doi:10.1074/jbc.274.23.16349, PMID:10347193.
- [155] Gerber HP, Dixit V, Ferrara N. Vascular endothelial growth factor induces expression of the antiapoptotic proteins Bcl-2 and A1 in vascular endothelial cells. *J Biol Chem* 1998;273(21):13313–13316. doi:10.1074/jbc.273.21.13313, PMID:9582377.
- [156] Gerber HP, Condorelli F, Park J, Ferrara N. Differential transcriptional regulation of the two vascular endothelial growth factor receptor genes. Flt-1, but not Flk-1/KDR, is up-regulated by hypoxia. *J Biol Chem* 1997;272(38):23659–23667. doi:10.1074/jbc.272.38.23659, PMID:9295307.
- [157] Wietecha MS, DiPietro LA. Therapeutic Approaches to the Regulation of Wound Angiogenesis. *Adv Wound Care (New Rochelle)* 2013;2(3):81–86. doi:10.1089/wound.2011.0348, PMID:24527330.
- [158] Witmer AN, Vrensen GF, Van Noorden CJ, Schlingemann RO. Vascu-

- lar endothelial growth factors and angiogenesis in eye disease. *Prog Retin Eye Res* 2003;22(1):1–29. doi:10.1016/s1350-9462(02)00043-5, PMID:12597922.
- [159] Ziyadeh FN. Different roles for TGF-beta and VEGF in the pathogenesis of the cardinal features of diabetic nephropathy. *Diabetes Res Clin Pract* 2008;82(Suppl 1):S38–S41. doi:10.1016/j.diabres.2008.09.016, PMID:18842317.
- [160] Vriese AS, Tilton RG, Elger M, Stephan CC, Kriz W, Lameire NH. Antibodies against vascular endothelial growth factor improve early renal dysfunction in experimental diabetes. *J Am Soc Nephrol* 2001;12(5):993–1000. doi:10.1681/ASN.V125993, PMID:11316858.
- [161] Natale P, Palmer SC, Jaurea A, Hodson EM, Ruospo M, Cooper TE, *et al*. Hypoxia-inducible factor stabilisers for the anaemia of chronic kidney disease. *Cochrane Database Syst Rev* 2022;8(8):CD013751. doi:10.1002/14651858.CD013751.pub2, PMID:36005278.
- [162] Gao W, Sun L, Fu X, Lin Z, Xie W, Zhang W, *et al*. Enhanced diabetic wound healing by electrospun core-sheath fibers loaded with dimethylolxylglycine. *J Mater Chem B* 2018;6(2):277–288. doi:10.1039/c7tb02342a, PMID:32254170.
- [163] Jeong W, Park SR, Rapisarda A, Fer N, Kinders RJ, Chen A, *et al*. Weekly EZN-2208 (PEGylated SN-38) in combination with bevacizumab in patients with refractory solid tumors. *Invest New Drugs* 2014;32(2):340–346. doi:10.1007/s10637-013-0048-3, PMID:24242862.
- [164] Schmidt KT, Huitema ADR, Dorlo TPC, Peer CJ, Cordes LM, Sciuto L, *et al*. Population pharmacokinetic analysis of nanoparticle-bound and free camptothecin after administration of NLG207 in adults with advanced solid tumors. *Cancer Chemother Pharmacol* 2020;86(4):475–486. doi:10.1007/s00280-020-04134-9, PMID:32897402.
- [165] Mu R, Li C, Li X, Ke Y, Zhao L, Chen L, *et al*. Effectiveness and safety of iguratimod treatment in patients with active rheumatoid arthritis in Chinese: A nationwide, prospective real-world study. *Lancet Reg Health West Pac* 2021;10:100128. doi:10.1016/j.lanwpc.2021.100128, PMID:34327344.
- [166] Gurova KV, Hill JE, Guo C, Prokvolit A, Burdelya LG, Samoylova E, *et al*. Small molecules that reactivate p53 in renal cell carcinoma reveal a NF-kappaB-dependent mechanism of p53 suppression in tumors. *Proc Natl Acad Sci U S A* 2005;102(48):17448–17453. doi:10.1073/pnas.0508888102, PMID:16287968.
- [167] Zhang F, Yan C, Wei C, Yao Y, Ma X, Gong Z, *et al*. Vinpocetine Inhibits NF-kB-Dependent Inflammation in Acute Ischemic Stroke Patients. *Transl Stroke Res* 2018;9(2):174–184. doi:10.1007/s12975-017-0549-z, PMID:28691141.
- [168] Tegtmeyer F, Schinzel R, Beer R, Bulters D, LeFrant JY, Sahuquillo J, *et al*. Efficacy of Ronoplerin (VAS203) in Patients with Moderate and Severe Traumatic Brain Injury (NOSTRA phase III trial): study protocol of a confirmatory, placebo-controlled, randomised, double blind, multicentre study. *Trials* 2020;21(1):80. doi:10.1186/s13063-019-3965-4, PMID:31937347.
- [169] Morató X, Marquí M, Tartari JP, Lafuente A, Abdelnour C, Alegret M, *et al*. A randomized, open-label clinical trial in mild cognitive impairment with EGb 761 examining blood markers of inflammation and oxidative stress. *Sci Rep* 2023;13(1):5406. doi:10.1038/s41598-023-32515-6, PMID:37012306.
- [170] Deev R, Plaksa I, Bozo I, Isaev A. Results of an International Post-marketing Surveillance Study of pI-VEGF165 Safety and Efficacy in 210 Patients with Peripheral Arterial Disease. *Am J Cardiovasc Drugs* 2017;17(3):235–242. doi:10.1007/s40256-016-0210-3, PMID:28050885.
- [171] Talman V, Kivelä R. Cardiomyocyte-Endothelial Cell Interactions in Cardiac Remodeling and Regeneration. *Front Cardiovasc Med* 2018;5:101. doi:10.3389/fcvm.2018.00101, PMID:30175102.
- [172] Cremolini C, Loupakis F, Antoniotti C, Lonardi S, Masi G, Salvatore L, *et al*. Early tumor shrinkage and depth of response predict long-term outcome in metastatic colorectal cancer patients treated with first-line chemotherapy plus bevacizumab: results from phase III TRIBE trial by the Gruppo Oncologico del Nord Ovest. *Ann Oncol* 2015;26(6):1188–1194. doi:10.1093/annonc/mdv112, PMID:25712456.
- [173] Gordon MS, Nemunaitis J, Barve M, Wainberg ZA, Hamilton EP, Ramanathan RK, *et al*. Phase I Open-Label Study Evaluating the Safety, Pharmacokinetics, and Preliminary Efficacy of Dilpacinib in Patients with Advanced Solid Tumors. *Mol Cancer Ther* 2021;20(10):1988–1995. doi:10.1158/1535-7163.MCT-20-0985, PMID:34315767.
- [174] Dugel PU, Koh A, Ogura Y, Jaffe GJ, Schmidt-Erfurth U, Brown DM, *et al*. HAWK and HARRIER: Phase 3, Multicenter, Randomized, Double-Masked Trials of Brolicizumab for Neovascular Age-Related Macular Degeneration. *Ophthalmology* 2020;127(1):72–84. doi:10.1016/j.ophtha.2019.04.017, PMID:30986442.
- [175] Wykoff CC, Abreu F, Adamis AP, Basu K, Eichenbaum DA, Haskova Z, *et al*. Efficacy, durability, and safety of intravitreal faricimab with extended dosing up to every 16 weeks in patients with diabetic macular oedema (YOSEMITE and RHINE): two randomised, double-masked, phase 3 trials. *Lancet* 2022;399(10326):741–755. doi:10.1016/S0140-6736(22)00018-6, PMID:35085503.
- [176] Olson E, Mahar KM, Morgan L, Fillmore C, Holland C, Lavery L. Randomized Phase I Trial to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of Topical Daprodustat in Healthy Volunteers and in Patients With Diabetic Foot Ulcers. *Clin Pharmacol Drug Dev* 2019;8(6):765–778. doi:10.1002/cpdd.654, PMID:30720931.
- [177] Chen MH, Wang YH, Sun BJ, Yu LM, Chen QQ, Han XX, *et al*. HIF-1 $\alpha$  activator DMOG inhibits alveolar bone resorption in murine periodontitis by regulating macrophage polarization. *Int Immunopharmacol* 2021;99:107901. doi:10.1016/j.intimp.2021.107901, PMID:34273637.
- [178] Wang Y, Cao Z, Wei Q, Ma K, Hu W, Huang Q, *et al*. VH298-loaded extracellular vesicles released from gelatin methacryloyl hydrogel facilitate diabetic wound healing by HIF-1 $\alpha$ -mediated enhancement of angiogenesis. *Acta Biomater* 2022;147:342–355. doi:10.1016/j.actbio.2022.05.018, PMID:35580827.
- [179] Lei Z, Klasson TD, Brandt MM, van de Hoek G, Logister I, Cheng C, *et al*. Control of Angiogenesis via a VHL/miR-212/132 Axis. *Cells* 2020;9(4):1017. doi:10.3390/cells9041017, PMID:32325871.
- [180] Kleinert M, Clemmensen C, Hofmann SM, Moore MC, Renner S, Woods SC, *et al*. Animal models of obesity and diabetes mellitus. *Nat Rev Endocrinol* 2018;14(3):140–162. doi:10.1038/nrendo.2017.161, PMID:29348476.
- [181] Baker RG, Hayden MS, Ghosh S. NF-kB, inflammation, and metabolic disease. *Cell Metab* 2011;13(1):11–22. doi:10.1016/j.cmet.2010.12.008, PMID:21195345.
- [182] Song W, Yin W, Ding L, Gao Y, Xu J, Yang Y, *et al*. Vinpocetine reduces cisplatin-induced acute kidney injury through inhibition of NF-kB pathway and activation of Nrf2/ARE pathway in rats. *Int Urol Nephrol* 2020;52(7):1389–1401. doi:10.1007/s11255-020-02485-z, PMID:32418008.
- [183] Abdelzaher WY, Ahmed SM, Welson NN, Marraiki N, Batiha GE, Kamel MY. Vinpocetine ameliorates L-arginine induced acute pancreatitis via Sirt1/Nrf2/TNF pathway and inhibition of oxidative stress, inflammation, and apoptosis. *Biomed Pharmacother* 2021;133:110976. doi:10.1016/j.biopha.2020.110976, PMID:33202281.
- [184] Chen S, Lv K, Sharda A, Deng J, Zeng W, Zhang C, *et al*. Anti-thrombotic effects mediated by dihydromyricetin involve both platelet inhibition and endothelial protection. *Pharmacol Res* 2021;167:105540. doi:10.1016/j.phrs.2021.105540, PMID:33711433.
- [185] Schwentker A, Vodovotz Y, Weller R, Billiar TR. Nitric oxide and wound repair: role of cytokines? *Nitric Oxide* 2002;7(1):1–10. doi:10.1016/s1089-8603(02)00002-2, PMID:12175813.
- [186] Edmonds ME, Bodansky HJ, Boulton AJM, Chadwick PJ, Dang CN, D'Costa R, *et al*. Multicenter, randomized controlled, observer-blinded study of a nitric oxide generating treatment in foot ulcers of patients with diabetes-PrOx1 study. *Wound Repair Regen* 2018;26(2):228–237. doi:10.1111/wrr.12630, PMID:29617058.
- [187] Jang MJ, Bae SK, Jung YS, Kim JC, Kim JS, Park SK, *et al*. Enhanced wound healing using a 3D printed VEGF-mimicking peptide incorporated hydrogel patch in a pig model. *Biomed Mater* 2021;16(4):5013. doi:10.1088/1748-605X/abf1a8, PMID:33761488.
- [188] Hicklin DJ, Ellis LM. Role of the vascular endothelial growth factor pathway in tumor growth and angiogenesis. *J Clin Oncol* 2005;23(5):1011–1027. doi:10.1200/JCO.2005.06.081, PMID:15585754.
- [189] Gainza P, Wehrle S, Van Hall-Beauvais A, Marchand A, Scheck A, Hartevelde Z, *et al*. De novo design of protein interactions with learned surface fingerprints. *Nature* 2023;617(7959):176–184. doi:10.1038/s41586-023-05993-x, PMID:37100904.