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Growth factors in the pathogenesis of diabetic foot ulcers

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TABLE OF CONTENTS

1. ABSTRACT

Foot ulcers affect 15% of patients with diabetes, resulting in a great health burden. The occurrence and development of diabetic foot ulcers is associated with neuropathy, peripheral arterial disease, and infection. Several growth factors are involved in these processes, including epidermal growth factor, vascular endothelial growth factor, transforming growth factor-beta, fibroblast growth factor, and erythropoietin, which could promote wound healing of patients with diabetes. Thus, this review discusses the role of these growth factors in the pathogenesis of diabetic foot ulcers, aiming to achieve novel insights into the management of diabetic foot ulcers.

2. INTRODUCTION

Diabetic foot ulcers are a common complication of diabetes, and they pose a great health burden among patients with diabetes. Foot ulcers may occur in approximately 15% of patients with diabetes during their lifetime and account for approximately 85% of all non-traumatic lower extremity amputations in these patients (1, 2). Diabetic foot ulcers are foot ulcerations associated with neuropathy and/or peripheral arterial disease of the lower limb in patients with diabetes. Peripheral neuropathy accounts for the majority of diabetic foot ulcers, followed by peripheral vascular disease (3). Diabetic neuropathy accounts for nearly 90% of neuropathy-induced ulcers (3). Motor neuropathy may lead to the atrophy and paresis of muscle, while sensory neuropathy may lead to the loss of protective sensation (4). Additionally, peripheral arterial disease is involved in the pathogenesis of diabetic foot ulcers, and is an independent predictor of the prognosis of the foot ulceration (5). An inadequate blood supply due to arterial disease may lead to local ischemia, necrosis, and subsequent infection in patients with diabetic foot ulcers (6). These processes may involve inflammation and infection (7). Thus, diabetic neuropathy and peripheral arterial disease are the basis of pathogenesis of diabetic foot ulcer. Peripheral neuropathy and peripheral vascular disease may be an effective therapy target of diabetic ulcers. During wound healing, extracellular matrix that has been remodeled by fibroblasts provides structural framework for the healing tissues (8). Growth factors related to peripheral neuropathy, vascular disease, inflammation, and extracellular matrix have gained increasing attention for their potential application in the treatment of diabetic foot ulcers.

Several growth factors are related to diabetic foot wound healing (9). For instance, connective tissue growth factor could improve the healing of diabetic foot ulcers through the accumulation of collagen IV and macrophages, and increasing wound α-smooth

muscle actins (10). Although growth factors such as platelet-derived growth factor may stimulate cell proliferation and survival (11), they do not show efficacy in clinical practice (12). In contrast, growth factors, including epidermal growth factor, vascular endothelial growth factor, transforming growth factor-beta, fibroblast growth factors, and erythropoietin have been demonstrated to have precise roles in the pathogenesis of diabetic ulcers. Therefore, this review discusses the role of these growth factors in the pathogenesis of diabetic foot ulcers, which may provide novel insights into the management of diabetic foot ulcers.

3. GROWTH FACTORS IN THE PATHOGENESIS OF DIABETIC FOOT ULCERS

3.1. Epidermal growth factor

Epidermal growth factor (EGF) stimulates cell growth, proliferation, differentiation, and survival by binding to its receptor EGFR (13). EGF with intralesional application was found to reduce amputations in 11 patients with diabetes with advanced foot ulcers (14). These patients had undergone several treatments, including revascularization, hyperbaric oxygen therapy, negative pressure wound therapy, and standard care. However, all these treatments failed to heal the ulcers, and amputation was the final choice (14). Recombinant human EGF (rhEGF) was used in these patients and showed a surprising effect on ulcer healing. This effect of EGF on promoting ulcer healing may be due to reducing oxidative stress and restoring the systemic redox balance of patients, in addition to stimulating cell growth and proliferation (15), because oxidative stress promotes non-healing diabetic foot ulcers (16).

The efficacy of EGF in the treatment of diabetic foot ulcers has been investigated in clinical studies. A randomized double-blinded controlled trial with 34 patients found that intralesional rhEGF could increase the rate of complete ulcer healing, promote epithelialization of the wound bed, and improve patient outcomes, whereas the side effect was mild transitory dizziness, which was considered acceptable (17). At approximately the same time, Singla et al. reported similar efficacy of rhEGF on Wagner's Grade 1 and 2 diabetic foot ulcers with 50 patients (18). They also found that rhEGF could reduce the healing time. Additionally, rhEGF has been found to be effective and safe in chronic diabetic foot ulcers (19). Although the samples of these previous studies were small, they demonstrate that EGF is effective and safe in the treatment of diabetic ulcers. Recently, a meta-analysis including four randomized controlled trials and 294 patients showed that rhEGF could increase the rate of wound healing, and that the rate of healing after rhEGF treatment was more than four times that of the controls (20). Therefore, EGF is a promising drug in diabetic ulcer treatment. In future, clinical trials with a large sample should be conducted to verify rhEGF's efficacy and safety in the treatment of diabetic ulcers.

3.2. Vascular endothelial growth factor

One of the risk factors for diabetic foot ulcers is peripheral arterial disease (21), which is also an independent predictor of poor outcome among patients with diabetes with ulcers (22). Vascular diseases may lead to local ischemia and necrosis during the development of diabetic foot ulcers and interrupt ulcer recovery. Thus, angiogenic factors could contribute to ulcer healing. Vascular endothelial growth factor (VEGF) is a protein that stimulates vasculogenesis and angiogenesis (23). The VEGF family comprises five members in mammals: VEGF-A to -D and placental growth factor (23). In patients with diabetic foot ulcers, a decreased level of VEGF-A was observed when compared with patients with diabetes without ulcers, and the inactivation of VEGF-A may lead to decreased levels of VEGF receptor-2 (24). Moreover, a decreased level of VEFG receptor-2 was proposed as a cause of poor wound healing (25). All these findings suggest an important role of VEGF in the recovery of diabetic ulcers. This was further confirmed by the findings of Amoli et al. that a lower frequency of genotype AA and A alleles in VEGF genes was associated with the occurrence of foot ulcers in diabetic patients (26). Therefore, VEGF has been treated as a biomarker of diabetic wound healing. Studies on the treatment of diabetic foot ulcers usually assess the efficacy by the level of VEGF or VEGF receptors (27–29).

Because VEGF is quite important in angiogenesis and diabetic wound healing, recombinant human VEGF (rhVEGF) has been used to treat diabetic ulcers. In 2008, a phase I trial of rhVEGF (telbermin) on chronic neuropathic diabetic foot ulcers was reported (30). This trial included a total of 55 patients with type 1 or 2 diabetes. Among them, 29 patients received telbermin and 26 received placebo. This study observed that telbermin had a tendency to increase the rate of complete ulcer healing and reduce the time to complete healing, but not significantly. The rhVEGF did not show significant efficacy when it was used alone in treating diabetic ulcers. This may have been due to the complex microenvironment of the ulcer and process of wound healing. In addition to peripheral arterial disease, neuropathy, inflammation, and infection may partly contribute to ulcer development. However, the study by Lois et al. provided novel insights on the use of rhVEGF for the treatment of diabetic ulcers. They found that fibrin-based scaffold incorporating VEGF- and fibroblast growth factor-2-loaded nanoparticles could promote wound healing in diabetic mice models (31). Thus, rhVEGF combined with other components may be a novel and effective

choice for treating diabetic ulcers. Further exploration is needed to determine which components might be appropriate for ulcer treatment. Other growth factors that promote wound healing or new biological materials may also be considered. For instance, extracellular matrix metalloproteinase inducer (namely CD147), which could be stimulated by advanced glycation end products and high glucose concentrations, and could induce VEGF in human retinal microvascular endothelial cells (32, 33).

3.3. Transforming growth factor-beta

Transforming growth factor-beta (TGF-β) is a pleiotropic growth factor that affects wound healing, and includes three isoforms (TGF-β1 to 3). It is secreted by inflammatory cells, such as macrophages, and then activates signaling that regulates cell differentiation or proliferation (34, 35). TGF-β/SMAD3 signaling is believed to participate in regulating glucose and energy homeostasis (36). Moreover, TGF-β may induce epithelial-mesenchymal transition (35), which is a crucial morphogenetic event in tissue formation and regeneration during wound healing (37, 38). A genetic polymorphism of the TGF-β gene with 74GG or 74GC genotypes has been found in chronic ulcers (39). Thus, TGF-β may be involved in the wound healing of diabetic ulcers. A study of skin biopsies found that TGF-β3 expression was increased in the epithelium at the edges of diabetic foot ulcers and venous ulcers compared with normal skin, whereas TGF-β1 expression was not changed (40). However, in diabetic mice, the expression of both TGF-β1 and TGF-β3 was increased (41). These findings demonstrated the role of TGF-β in the healing of diabetic ulcers. Later, findings from a clinical study further confirmed this role of TGF-β1. In that study, a decreased level of TGF-β1 in ulcers was related to the prolonged wound healing of diabetic patients, and an increased level of TGF-β1 (>115 pg/ml) may be a predictor of healing within 12 weeks (42). However, although TGF-β1 could promote the migration of fibroblast cells into diabetic wounds through targeting the NFκB-miR-21 pathway in a high-glucose environment (43), the fibroblasts in patients with diabetes with foot ulcers may abnormally respond to TGF-β, resulting in non-healing ulcers (44). Thus, the response of fibroblasts to TGF-β and its related mechanism requires further study in patients with non-healing diabetic ulcers.

3.4. Fibroblast growth factors

Fibroblast growth factors (FGFs) are a family of growth factors that can directly regulate cell proliferation, migration, and differentiation (45). It could promote the proliferation of endothelial cells and their organization into tube-like structures, resulting in angiogenesis (45). In diabetic ulcers, FGF-2, also known as basic fibroblast growth factor, is related to wound healing (46). FGF-2 is thought to mediate angiogenesis during wound healing (47). During the wound healing of patients with diabetic foot ulcers, a significant upregulation of FGF-2 was observed, accompanied by an increased level of phosphorylated extracellular signal-regulated *protein* kinases 1 and 2 (ERK1/2) protein (48), suggesting that FGF-2 may perform its function on wound healing through ERK1/2 signaling. Although FGF-2 has been accepted as a crucial factor in diabetic wound healing, there are few reports on its usage in the treatment of diabetic ulcers. A study on the treatment of periodontal intra-bony defects showed that although recombinant human FGF-2 could promote wound regeneration, its efficacy was not satisfactory on clinical attachment level gain and gingival recession (49). Thus, the use of FGF-2 alone in wound treatment may not have satisfactory efficacy. However, combining FGF-2 with other drugs may be an effective method of treating diabetic ulcers.

3.5. Erythropoietin

Erythropoietin (EPO) is an essential hormone for stimulating erythropoiesis. It could also stimulate angiogenesis, and promote cell survival in ischemic tissues (50). The receptor of EPO is located on endothelial cells and neurons (51, 52). Thus, the effect of EPO on endothelia and nerves may be related to diabetic ulcers, which could be induced and aggravated by vascular diseases and neuropathy. In a study of small fiber neuropathy in a mouse model, recombinant human EPO (rhEPO) showed a beneficial effect on nerve and restored skin protective capacities against ischemic pressure (53), suggesting that EPO may contribute to the treatment of diabetic neuropathy. The rhEPO protects skin from pressure ulcers and prevents neuropathic diabetic ulcers through improving pressure-induced vasodilatation and the restoration of C-fiber nociception and skin innervation density (54).

In addition to the prevention of diabetic ulcers, EPO could promote ulcer healing. In the diabetic ulcer model, EPO not only promotes angiogenesis, but also stimulates mesenchymal stem cells to proliferate, migrate, and secrete VEGF even in a microenvironment with a high glucose level (55). At the same time, EPO inhibits the mesenchymal stem cells from secreting tumor necrosis factor alpha and inhibits monocyte to invade to ulcers, contributing to reducing local inflammation (55) (Figure 1). In diabetic patients with foot ulcers, a high-glucose microenvironment is common, and may promote the development of ulcers. Thus, EPO's ability to promote ulcer healing in a high-glucose microenvironment is a valuable characteristic. Although there has been no evidence of the efficacy of EPO in the treatment of patients with diabetic foot ulcers, rhEPO was reported to improve the outcome of patients with spinal cord injuries and pressure ulcers in a pilot study (56). Thus, the efficacy of rhEPO in the

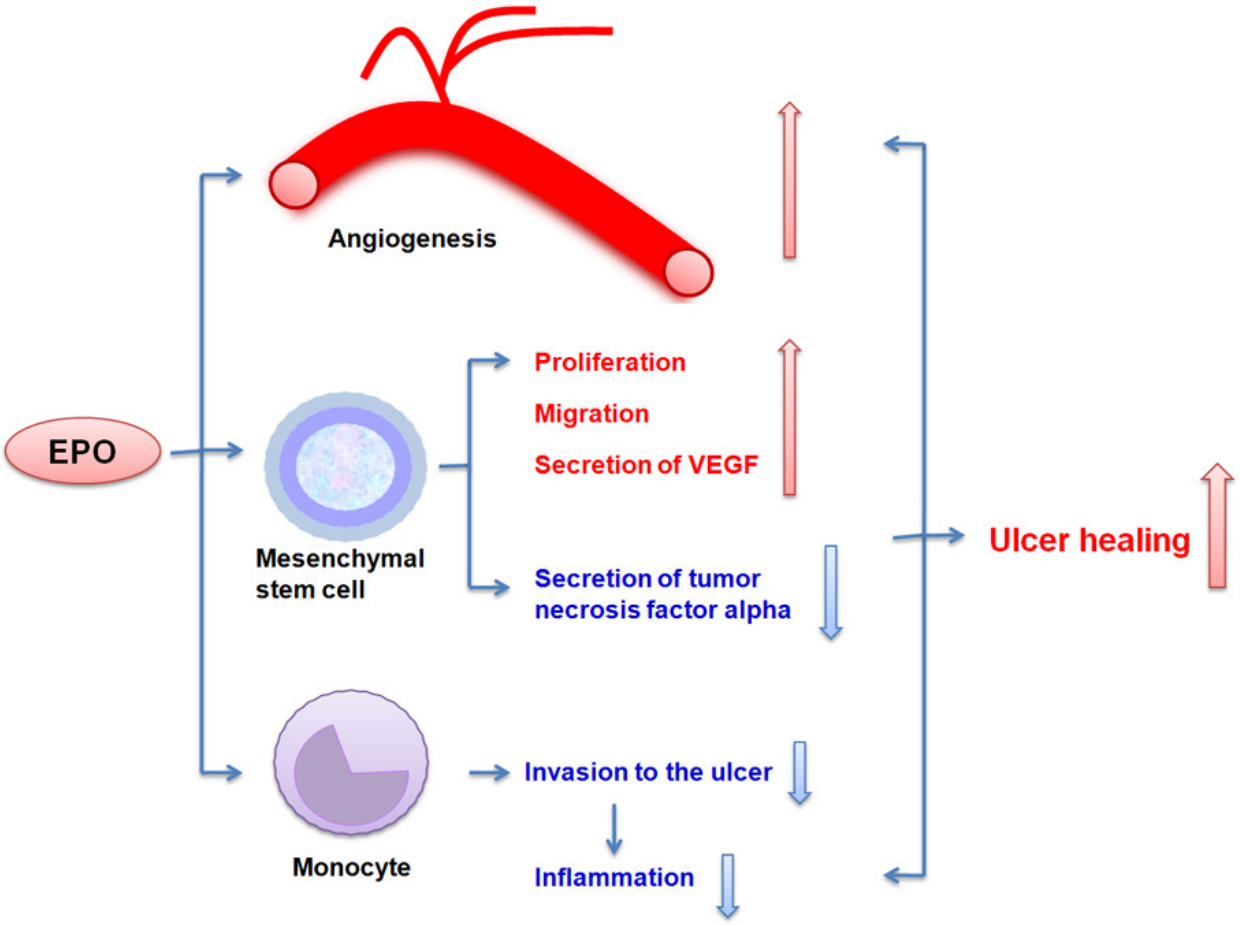

Figure 1. EPO promotes the healing of diabetic foot ulcers. In diabetic foot ulcers, EPO not only promotes angiogenesis, but also targets mesenchymal stem cells and monocytes. It stimulates mesenchymal stem cells to proliferate, migrate, and secrete VEGF, whereas it inhibits the stem cells from secreting tumor necrosis factor alpha. EPO also inhibits monocytes from invading ulcers, resulting in decreased local inflammation. EPO: erythropoietin; VEGF: endothelial growth factor.

treatment of diabetic ulcers should be explored in future clinical studies.

4. CONCLUSIONS

In conclusion, growth factors, such as EGF, VEGF, TGF-β, FGF-2, and EPO, play a role in promoting healing during the pathogenesis of diabetic ulcers. rhEGF and rhEPO alone may be effective in treating diabetic ulcers, while rhVEGF and FGF-2 may need to be combined with other components. The efficacy of these growth factors in clinical practice requires further exploration in the future, which may provide a novel management strategy of diabetic foot ulcers.

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Abbreviation: EGF: epidermal growth factor; EGFR: epidermal growth factor receptor; rhEGF: recombinant human EGF;VEGF: endothelial growth factor; rhVEGF: recombinant human VEGF;TGF-β: transforming growth factor-beta; FGF: fibroblast growth factor; EPO: erythropoietin; rhEPO: recombinant human EPO

Key Words: Growth factors, Epidermal Growth Factor; Diabetic Foot Ulcers, Review

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