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DOI: 10.1177/1534734614520704

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What is This?
Promising Role of ANGPTL4 Gene in Diabetic Wound Healing

Awadhesh K. Arya, PhD¹, Kamlakar Tripathi, MD², and Parimal Das, PhD¹

Abstract

Diabetes mellitus (DM) is one of the severe metabolic disorders of carbohydrate metabolism worldwide. Developing countries are at higher risk of DM, and there is significant evidence that it is epidemic in many economically developing and newly industrialized countries. Among all other complications associated with DM, delayed wound healing is a major concern in diabetic patients. Wound healing is a natural healing process that starts immediately after injury. This involves interaction of a complex cascade of cellular events that generates resurfacing, reconstitution, and restoration of the tensile strength of injured skin. There are multiple factors responsible for delayed wound healing among which the contribution of DM has been well documented. The wound healing process is also delayed by the metabolic, vascular, neurological, and inflammatory alterations, which are well known in both type 1 and type 2 diabetes. Keratinocytes are crucial for wound re-epithelialization, and defects in directed migration of keratinocytes due to DM are associated with the delayed wound healing process. Many factors responsible for re-epithelialization have been identified, characterized, and well described; however, the genes responsible for the healing process have only partially been illustrated. This article will therefore focus on the efficacy of ANGPTL4 (angiopoietin-like 4) gene, which plays a novel role in keratinocyte migration during wound healing.

Keywords
diabetes mellitus, wound, gene, keratinocyte

Diabetes mellitus (DM) is one of the most frequent chronic diseases in nearly all countries and continues to increase in numbers at epidemic proportions worldwide. The major factor responsible for DM is changing lifestyle, which includes reduced physical activity and increased obesity.¹ According to International Diabetes Federation, 366 million people were suffering from diabetes in 2011, which is expected to increase to 552 million by 2030 worldwide. In India, 61.3 million people were suffering from DM in 2011, which is expected to become 101.2 million in 2030.² These data suggest that diabetes is undoubtedly one of the most challenging health problems in the 21st century. Prolonged diabetes leads to various complications and is a major cause of disability, reduced quality of life, and death. Diabetes complications can affect various parts of the body apparently in different ways for different people. One of the most apparent and devastating complications of diabetes is the development of chronic nonhealing foot ulcerations, occurring in 15% of diabetics,³ 66% of non-traumatic lower-extremity amputations, and 30% to 45% of renal failure cases requiring renal replacement therapy.⁴ Delayed healing of chronic nonhealing ulcer in diabetic patients also enhances the possibility of developing coronary artery disease.⁵

There are multiple factors responsible for impaired wound healing in DM, relating both to impaired glucose metabolism and to the effect of neurovascular complications. It has been postulated that hyperglycemia itself has a deleterious effect on wound healing through the formation of reactive oxygen species and enhanced apoptosis of lymphocytes.⁶ An altered immune function may also contribute to poor wound healing in patients with DM.⁷

An essential feature of a healed wound is the restoration of an intact epidermal barrier through wound epithelialization, also known as re-epithelialization. In DM patients, re-epithelialization is disrupted, supposedly due to higher blood sugar.⁸ There are many genes reported those are responsible for

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re-epithelialization such as expression of primate-specific exonic microRNA-198 (miR-198), located in the 39-untranslated region of follistatin-like 1 (FSTL1) messenger RNA. miR-198 switches to expression of the linked open reading frame of FSTL1 during wounding in a human ex vivo organ culture system. Sundaram et al showed that failure of FSTL1 (pro-migratory) expression and last miR-198 (anti-migratory) expression is evident in nonhealing chronic diabetic ulcers. In this condition, keratinocyte migration, re-epithelialization, and wound healing all fail to occur. Viticchie et al also suggest that miR-203 controls the expression of target proteins those are responsible for both keratinocyte proliferation and migration during wound re-epithelialization. In addition to the role of microRNA, Stanniocalcin-1 (STC1) regulates re-epithelialization in human keratinocytes, and α3β1-integrin-controlled Smad7 regulates re-epithelialization in mice during wound healing.

There are several reports published that show DM alters the proper functioning of the genes responsible for re-epithelialization, which may be the reason for delayed wound healing in diabetic patients. Among all genes, in present review we will focus on the studies showing the importance of ANGPTL4 (angiopoietin-like 4) gene in wound healing.

**Overview of ANGPTL4**

Angiopoetin-like protein (ANGPTL) was discovered in 2000 by 3 independent research groups as a new protein that was similar to members of the ANG (angiopoietin) family. The HUGO Gene Nomenclature Committee has named the gene encoding PPARγ/ANG-related protein as ANGPTL4 (ANG-like 4). PPARγ/ANG-related protein is a downstream target of PPARα (peroxisome-proliferator-activated receptor α) and PPARγ during pre-adipocyte differentiation. ANGPTL4 protein has multiple functions due to its production from various parts of body such as liver, adipose tissue, blood, pancreas, kidney, intestine, brain, placenta, and skin. ANGPTL4 protein has a role in re-epithelialization during wound healing and also plays an incredibly significant role in lipid and glucose metabolism.

Plasma glucose concentration in type 2 DM patients is higher whereas ANGPTL4 concentration in these patients is lower, showing inverse correlation between glucose concentration and ANGPTL4. The efficiency of ANGPTL4 to lower glucose concentration might be partly mediated by its direct inhibition of hepatic glucose output. The advantageous effects of ANGPTL4 on glucose homeostasis are related with detrimental hyperlipidemia and fatty liver in mice.

The role of ANGPTL4 in vascular and tumor processes is more deliberated and indicative of a context- and tissue-specific activity. A study by Verine et al shows that ANGPTL4 mRNA expression allows the discrimination of the renal origin of metastases from clear-cell carcinomas arising from various organs. ANGPTL4 is upregulated at both the protein and mRNA levels under hypoxic conditions, and its role in protection from atherosclerosis development and progression is well studied by ANGPTL4 knockout mice. Its role in pancreas acinus apoptosis is also well known, wherein overexpression of ANGPTL4 in the rat pancreas induced apoptosis of the acinus of the pancreas by influencing the BCL-2 level. Augmented expression of ANGPTL4 leads to decreased BCL-2 protein expression, which is achieved by suppression of ERK1/2-MAK pathway and TPK-Ras-MAK pathway inducing pancreas acinus apoptosis.

**Genetic Assembly and Expression of ANGPTL4**

ANGPTL4 is a protein that is encoded by the ANGPTL4 gene in humans. The size of human ANGPTL4 gene is 10.25 kb, located between nucleotides 8 429 011 and 8 439 257 on chromosome 19p13.3 having 7 exons and encodes a 406-amino-acid glycoprotein with a molecular mass of ≈45 to 65 kDa (see Figure 1).

ANGPTL4 is a member of the angiopoietin/angiopoietin-like gene family and encodes a glycosylated, secreted protein with a coiled-coil N-terminal domain and a fibrinogen-like C-terminal domain. Angiopoietin-like gene family
having 7 members denoted as ANGPTL1-7 present in both humans and mice except for ANGPTL5, which is a human orthologue. ANGPTL4 protein exists as an oligomer containing intermolecular disulfide bonds. Oligomerized ANGPTL4 undergoes proteolysis to release its C-terminal fibrinogen-like domain, which circulates as a monomer. The function of cANGPTL4 is still comparatively uncertain, but it has been assumed to be involved in the persistence of vascular endothelial veracity. The higher order oligomeric structure of ANGPTL4 is mediated by oligomerization of N-terminal coiled-coil domain, which shows its importance in LPL (lipoprotein lipase) inhibition. In hepatocytes and white adipose tissue triglyceride, lipid metabolism and ANGPTL4 gene expression are upregulated during fasting conditions. Glucocorticoids are reported as an inducer of ANGPTL4 expression during fasting where ANGPTL4 directly increase the level of cAMP and the phosphorylation of downstream TG hydrolytic enzymes. Overexpression of ANGPTL4 increases the uptake of fatty acids and cholesterol into tissues from blood circulation. These findings demonstrate a novel property of ANGPTL and suggest that oligomerization and proteolytic processing of ANGPTL4 may regulate its biological activities in vivo.

Several studies showed the association of ANGPTL4 with various diseases. Mikhail et al observed an association between the minor allele (A) for ANGPTL4 rs11672433 and haplotypes carrying this allele with risk of brain arteriovenous malformations but not with intracranial hemorrhage presentation.

### Basic Characteristics of Wound Healing

Wound healing is a natural process; it is a well-orchestrated integration of the complex biological and molecular events of cell migration, cell proliferation, and extracellular matrix (ECM) deposition. Normal wound healing can be divided into 4 overlapping phases: hemostasis, inflammation, proliferation, and remodeling.

#### Hemostasis

Hemostasis is the first step of wound healing. It is a process to stop blood flow from damaged blood vessels. It involves 3 major steps: (a) vasoconstriction, (b) temporary blockage of a break by a platelet plug, and (c) blood coagulation, or formation of a clot that seals the opening until tissues are repaired. Clots formed during hemostasis are primarily composed of embedded blood cells in the fibrin mesh and aggregated platelets. The formation of clot is very important to prevent further fluid and electrolyte loss from the wound site and limits contamination from the outside environment.

#### Inflammation

Inflammation is characterized by increased vascular permeability and the sequential migration of inflammatory cells like leukocytes into the extravascular space of injured tissue. Due to the damage of blood vessels during injury, the wounds undergo a hypoxic condition, which increases keratinocyte migration, early angiogenesis, proliferation, and clonal expansion of fibroblasts. Inflammatory cells destroy bacteria and eliminate debris from dying cells and damaged matrix so that the repair processes can proceed at the wound site. Neutrophils and macrophages play a major role in the debridement, and their function is impaired in diabetes. Angiogenesis, fibroblast migration and proliferation, and collagen production are facilitated by the cytokines produced from macrophage and are possibly involved in wound contraction. TGF-β, IL-1, insulin-like growth factor-1 (IGF-1), FGF-2, and PDGF are some other cytokines derived from macrophage. Lymphocytes play a crucial role in the wound healing process as well, and the early apoptosis of lymphocytes delays wound healing in diabetes.

#### Proliferation

The proliferative phase starts after 4 days of wound and is characterized by angiogenesis, collagen deposition, granulation tissue formation, epithelialization, and wound contraction. It lasts until day 21 in acute wounds, depending on the size of the wound and the health of the patient. In angiogenesis, new blood vessels are formed by vascular endothelial cells, which makes possible the resupply of oxygen and other nutrients, whereas persistent utility and reliability of injured tissues are maintained by matrix proteins including collagens, fibronectin, and vitronectin, which provide substrates for cell movement. The processes of cellular migration and proliferation occur under the control of various cytokines including EGF, TGF-α, platelet-derived EGF, and keratinocyte growth factor. Wound contraction is speeded up by the formation of ECM, granulation tissue, and the emergence of myofibroblasts at the wound site.

#### Remodeling

At the last stage of healing process, remodeling and realignment of the collagen tissue to produce greater tensile strength to wound takes place. During this process collagen synthesis diminishes and reaches coincides with the rate of collagen breakdown, which is influenced mainly due to matrix metalloproteinases. With the progress of the remodeling phase, the tensile strength of the wound increases and achieves 50% strength of normal tissue by 3 months after injury, ultimately becoming as much as 80% as strong as normal tissue.
**Expediency of ANGPTL4 in Wound Healing**

Normal wound healing is a multifaceted cellular comeback that relies on the timed release of various signaling molecules to coordinate the activities of inflammatory cells, endothelial cells, keratinocytes, and fibroblasts.41 DM influences nearly all participating cells and expression of cytokines and growth factors required during the wound healing process.3 Impaired wound healing in diabetic patients is also associated with the accumulation of advanced glycated end products in the tissues. It is the result of nonenzymatic reaction between the amino groups of proteins and reducing sugars. Proteins such as collagen and fibronectin are present in ECM and reducing sugars are abundant in tissues under hyperglycemic condition.42 These modifications of the ECM proteins alter cell–ECM interactions, including cell adhesion and migration that are critical for wound healing.42 Cell migration during wound healing is activated by binding of ANGPTL4 with integrin-β1, which further activates the FAKSrc-PAK1 signaling pathway. The modulation of keratinocyte migration is also mediated by ANGPTL4 protein mainly by 2 ways. First, cell migration is facilitated by integrin-mediated signaling whose effectiveness is increased by ANGPTL4. Subsequently, depending on the local context of the ECM, ANGPTL4-bound integrins provide a novel means by which selective integrin signaling cascades can be activated. Diminished integrin-mediated signaling delays wound re-epithelialization with impaired keratinocyte migration.43 Keratinocyte migration starts after 24 ± 48 hours form the cut edge of the wound. It travels throughout the wound bed not only from the wound margin but also from cut epidermal attachments.44 The migrating keratinocytes have more prominent gap junctions but lose hemidesmosomes and desmosome.45 During this process keratin filaments withdraw from the cytoplasmic margin and there is relocation of the actin cytoskeleton into the lamellipodia.46

ANGPTL4 regulates the availability of the local ECM in the wound bed by interacting with vitronectin and fibronectin. This interaction delays the degradation of these proteins by proteases. The delayed degradation of vitronectin and fibronectin stabilize the integrin–matrix protein complex by providing a separate binding site for ANGPTL4. ANGPTL4 plays a novel role in wound healing by upregulating PPARβ/δ, which modifies the wound microenvironment to coordinate cell–matrix communication. PPARβ/δ is an important regulator of keratinocyte survival in the wounded epidermis and is involved in cell adhesion and migration.47 PPARβ/δ regulates IL-1 signaling in dermal fibroblasts to control homeostatic of keratinocyte proliferation48 and stimulates epidermal differentiation during re-epithelialization of wound healing.49 Pal et al showed that PPARβ/δ mediated upregulation of ANGPTL4 expression in human keratinocytes stimulates the expression of protein kinase C and activities of activator protein-1 transcription factors to modulate epidermal differentiation.50

**Conclusion**

Impaired wound healing in diabetic patients is a state that influences a person to an elevated rate of medical complications leading to major social and economic burden on their life due to which their survival rates are reduced; therefore, wound healing is a well-studied research field over the past 2 decades. The main area of research has focused on understanding the physiology of normal wound healing and the pathophysiology of chronic wounds, such as diabetic foot ulcers. There are also many studies available on the efficacy of growth factors, cytokines, and chemokines, which play a major role in wound healing and affected during hyperglycaemic conditions.

Presently active research is focused on the re-epithelialization step of wound healing, which makes outer covering and provides strength to the wound. DM badly affects the re-epithelialization step of wound healing, and we have proposed that the study of the genes responsible for re-epithelialization can be better for the future therapeutic approach in proper wound healing. Among all other genes reported, ANGPTL4 shows a potential effect on lipid homeostasis, glucose metabolism, re-epithelialization, inflammation, and potential effect on energy homoeostasis, which is required for wound healing. These findings would be useful for further study on the expression and effects of ANGPTL4 for the development of therapies for normal wound healing and will hopefully promote wound healing to levels that are observed in healthy, nondiabetic subjects.

**Declaration of Conflicting Interests**

The author(s) declared no potential conflicts of interest with respect to the authorship, and/or publication of this article.

**Funding**

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: Awadhesh K. Arya would like to thank the University Grants Commission, India, for providing the Dr D.S. Kothari postdoctoral fellowship.

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