High-glucose environment increased thrombospondin-1 expression in keratinocytes via DNA hypomethylation

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Diabetes is an important health issue because of its increasing prevalence and association with impaired wound healing. Epidermal keratinocytes with overexpressed antiangiogenic molecule thrombospondin-1 (TSP1) have been shown to impair proper wound healing. This study examined the potential involvement of keratinocyte-derived TSP1 on diabetic wound healing. Cultured human keratinocytes and diabetic rat model were used to evaluate the effect of high-glucose environment on TSP1 expression in epidermal keratinocytes, and the molecular mechanisms involved in the process were also studied. We demonstrated that high-glucose environment increased TSP1 expression in keratinocytes. In addition, increased oxidative stress induced DNA hypomethylation at the TSP1 promoter region in keratinocytes exposed to high-glucose environment. Similar findings were found in our diabetic rat model. Early antioxidant administration normalized TSP1 expression and global DNA methylation status in diabetic rat skin and improved wound healing in vivo. Because oxidative stress contributed to TSP1 DNA hypomethylation, early recognition of diabetic condition and timely administration of antioxidant are logical approaches to reduce complications associated with diabetes as alterations in epigenome may not be reversible by controlling glucose levels during the later stages of disease course. (Translational Research 2016;169:91–101)

Abbreviations: TSP1 = thrombospondin-1; AGE = advanced glycation end-product; BSA = bovine serum albumin; 5-Aza-dC = 5-Aza-2′-deoxycytidine; MSP = methylation-specific PCR; ROS = reactive oxygen species; PBS = phosphate-buffered saline; PCR = polymerase chain reaction; Rluc = Renilla- Luciferase; TE = Tris-EDTA; STZ = streptozotocin; DAB = 3,3′-diaminobenzidine tetrahydrochloride; DM = diabetes mellitus; VEGF = vascular endothelial growth factor; NADPH = nicotinamide adenine dinucleotide phosphate; PVDF = polyvinylidene difluoride membrane; dUTP = 2′-deoxyuridine, 5′-triphosphate; cy5 = cyanine5 flurophore; SDS = sodium dodecyl sulphate; mDIP = methylated DNA immunoprecipitation; tRNA = transfer ribonucleic acid

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AT A GLANCE COMMENTARY

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Background

Diabetes is an important health issue because of its increasing prevalence and association with the development of serious complications include impaired wound healing.

Translational Significance

Present study demonstrated that high-glucose environment increased thrombospondin-1, an anti-angiogenic molecule that contributes to impaired wound healing, in epidermal keratinocytes. DNA hypomethylation at thrombospondin-1 promoter region because of increased oxidative stress is responsible for this event. These results provide a rationale explaining why impaired wound healing is commonly observed in diabetic patients, even in those with recent good blood glucose control and suggest new therapeutic strategy to improve diabetic healing.

INTRODUCTION

Wound healing is a carefully orchestrated process involving coagulation, inflammation, tissue formation, and tissue remodeling.[1] Impaired skin wound healing is a frequent cause of morbidity and mortality among patients with diabetes.[2] Diabetic wounds often pose a high risk for subsequent infection and amputation.[3] In fact, the rate of nontraumatic lower extremity amputation for diabetic patients is >40 times the rate for individuals who do not have diabetes.[4] Dysregulation of glucose homeostasis and elevated glucose level in the microenvironment are the central etiologies of diabetes.[5] Because the prevalence of diabetes is expected to increase around the globe,[6] understanding the mechanisms responsible for impaired diabetic wound healing may lead to the development of innovative and effective therapeutic strategies for treating this commonly encountered clinical disorder.

Previous studies on diabetic wound healing focused on various cellular populations involved in healing including inflammatory cells,[7,8] fibroblasts,[9,10] and endothelial cells.[11,12] The impact of diabetes on keratinocytes, the initiator of healing on skin barrier disruption, has not been carefully examined. Our previous studies demonstrated that the physiological functions of keratinocytes are hampered at high-glucose environment.[13,14] One of the hallmarks associated with impaired wound healing in diabetic condition is reduced angiogenesis. Although epidermal keratinocytes regulate dermal angiogenesis during wound healing, how high-glucose environment modulates angiogenesis via keratinocytes after wounding remains elusive.

The concept of balance between endogenous angiogenesis inhibitor vs angiogenesis stimuli has been proposed to control the vascular homeostasis of the skin. Thrombospondin (TSP), an angiogenesis suppressor, is a key molecule controlling the skin angiogenesis.[15] TSP is a family of matricellular proteins that mediate the interactions between extracellular matrix molecules and cellular integrin receptor.[16] TSP1, in particular, is important in maintaining vascular homeostasis of the skin. More specifically, TSP1 is a 450-kDa modular homotrimeric glycoprotein, containing a procollagen homology region, 3 properdin-like type I repeats, 3 epidermal growth factor–like repeats, and 7 Ca-binding repeats. TSP1 is involved in many physiological processes including suppression of angiogenesis.[17] In normal human skin, TSP1 is expressed by the basal epidermal keratinocytes and contributes to the barrier that prevents the growth of blood vessels into the dermis. On the other hand, the TSP1 expression of cutaneous squamous cell carcinoma is reduced, contributing to the angiogenesis and invasiveness of malignant cells.[18] In the context of wound healing, TSP1 has been shown to play a significant role. It has been demonstrated that transgenic mice with keratinocytes overexpressing TSP1 showed delayed healing of skin wounds associated with diminished angiogenesis.[19] This scenario is similar to the suboptimal healing frequently observed in diabetic condition.

Although keratinocyte-derived TSP1 is a critical regulator of angiogenesis during wound healing, the role of keratinocyte-derived TSP1 during diabetic wound healing remained unclear. It was shown that diabetic patients have increased TSP1 in their plasma and kidneys.[20] In addition, the TSP1 expressions were increased in the macrovessels of diabetic rats.[21] When cultivated under high-glucose environment, the mesangial cells showed increased TSP1 expression, and it has been demonstrated that TSP1 promoter region contains a glucose response element.[22,23] In the context of healing, it was shown that during spinal cord injury, the diabetic rats showed significantly higher TSP1 expression as compared with their nondiabetic counterparts, and altered local vascular regeneration was suggested to contribute to the more severe damage observed in diabetic rats.[24] Therefore, we hypothesized that high-glucose environment may increase TSP1 expression in the epidermal keratinocytes, an event that can contribute to suboptimal wound healing