ORIGINAL ARTICLE

Overexpression of miR-29b reduces collagen biosynthesis by inhibiting heat shock protein 47 during skin wound healing

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Skin scar formation is characterized by excessive synthesis and aberrant deposition of collagens during wound healing. MicroRNAs are endogenous gene regulators critically involved in diverse biological events including skin scar formation and hold considerable promise as therapeutic targets. However, the detailed molecular mechanisms responsible for collagen production during skin wound repair and scar formation remain incompletely known. Here our data revealed that significant downregulation of miR-29b and upregulation of heat shock protein 47 (HSP47) were observed during wound healing in both excisional and burn wound models and also detected in facial skin scar as compared to adjacent healthy skin. HSP47, a specific chaperon for collagen production and secretion, was identified as a novel and direct post-transcriptional target of miR-29b in skin fibroblasts via bioinformatics prediction and experimental validation. Moreover, the regulatory functions of miR-29b in collagen biosynthesis are partially achieved through modulating HSP47 expression in skin fibroblasts. Furthermore, the profibrotic growth factor TGF-β1 inhibited miR-29b transcription by activating TGF-β/Smads signaling and in turn depressed HSP47 and enhanced collagen 1 production. In contrast, the proinflammatory cytokines IL-1β and TNF-α significantly induced miR-29b transcription via activating NF-κB signaling but had no significant effect on HSP47 and collagen production in skin fibroblasts. Importantly, local delivery of miR-29b lentiviral particles inhibited HSP47 expression and collagen biosynthesis as well as suppressed angiogenesis, thus reducing scar formation in an excisional wound splinting model. Collectively, our data reveal that miR-29b can reduce collagen biosynthesis during skin wound healing likely via post-transcriptional inhibition of HSP47 expression. These findings also suggest that therapeutic targeting of miR-29b/HSP47 might be a viable alternative strategy to prevent or reduce scar formation. (Translational Research 2016;■:1–16)

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Abbreviations: ECM = extracellular matrix; HSP47 = heat shock protein 47; miR-29 = microRNA-29; 3′-UTR = 3′ untranslated region; IL-1β = interleukin-1 beta; TNF-α = tumor necrosis factor-alpha; TGF-β = transforming growth factor-beta; PDGF = platelet-derived growth factor; immunohistochemical staining = IHC; immunofluorescence = IF; VEGF = vascular endothelial growth factor

AT A GLANCE COMMENTARY

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Background

Skin scar formation is characterized by excessive synthesis and aberrant deposition of collagens during wound healing. MicroRNAs are endogenous gene regulators underlying diverse biological events including skin scar formation and hold considerable potentials as therapeutic targets. However, the detailed molecular mechanisms driving aberrant collagen production during skin wound repair remain incompletely known.

Translational Significance

Our findings had identified HSP47 as a direct target of miR-29b in skin fibroblasts and indicated that miR-29b can reduce collagen biosynthesis during skin wound healing likely via post-transcriptional inhibition of HSP47 expression. These findings also suggest that therapeutic targeting of miR-29b/HSP47 might be a viable alternative strategy to prevent or reduce scar formation.

INTRODUCTION

The skin wound healing is one of the most complex biological processes composed of 3 distinct but overlapping phases including inflammation, proliferation, and remodeling. This process is tightly orchestrated by various types of cells, cytokines, and growth factors in a sequential and coordinated manner.[1,2] On injury, diverse cells including inflammatory cells, keratocytes, and fibroblasts become activated and synchronized to restore tissue integrity. Compromised healing results in chronic wound or dehiscence, whereas normal healing in adults usually lead to scar formation that is characterized by excessive nonfunctioning disorganized deposition of extracellular matrix (ECM) components, especially the different types of collagens.[3] Particularly, the hypertrophic scar and keloid have several similar histologic features including thick and disorganized collagen bundles, along with increased angiogenesis, although they might have genetic predisposition.[4,5] Scar formation usually leads to functional disability, cosmetic deformities, psychological stress, and huge socioeconomic burden. Thus, the prevention and management of skin scar are continuing to be an unmet challenge for clinicians for decades largely due to the lack of effective treatments.[6]

It is well established that ECM metabolism such as collagen biosynthesis is the key cellular and molecular event underlying both normal and pathological skin wound healing, which greatly dictates the healing quality and outcome.[7] Mounting evidence has demonstrated that collagen biosynthesis and deposition by skin fibroblasts is intricately governed by multiple cytokines and growth factors including TGF-β1,TNF-α, and platelet-derived growth factor during wound repair.[8] However, the detailed mechanisms driving excessive collagen biosynthesis during healing and scarring remain incompletely known. Therefore, deciphering the regulatory circuit underlying collagen biosynthesis during wound healing might provide valuable insights into scar pathogenesis and identify potential therapeutic targets for scar management in the clinic.

Recent evidence has revealed that heat shock protein 47 (HSP47), encoded by gene *SerpinH1*, serves as the specific chaperon for collagen folding and secretion via transiently interacting in the endoplasmic reticulum.[9] Direct binding between HSP47 and procollagens is critically required for subsequent assembly, secretion, cleavage, and fibril formation of collagen.[10,11] Our previous studies and others have provided compelling evidence that HSP47 is critically involved in tissue fibrosis and scar formation. Highly elevated HSP47 was detected during skin scar formation, whereas much less of HSP47 was found during fetal scar less wound healing.[12] Inhibition of HSP47 by antisense oligonucleotides or siRNA reduced skin scar formation and fibrosis largely via reducing collagen production, whereas its enforced overexpression attenuated the deleterious effects of diabetes on cutaneous wound healing by enhancing collagen biosynthesis.[13-15] However, the in-depth understanding about the biological roles of HSP47 during skin wound healing, especially its own regulation underlying scar formation remains largely unexplored thus far.

MicroRNAs (miRNAs) are a novel class of endogenous RNAs that play pivotal roles in diverse physiological and pathologic contexts including skin development and wound healing.[16-18] They function through recognizing and binding complementary to the 3′ untranslated region (3′-UTR) of cognate mRNAs, leading to either mRNA degradation or