The synthetic peptide, containing the syndecan-binding sequence within laminin-3 chain module 4 (KN8FMALYL8KGRLVFALG) and the synthetic peptide A3G756 were shown to stimulate keratinocyte migration. In the present study, we demonstrate the usefulness of the A3G756 peptide for skin wound healing. First, we show that SB203580, the selective inhibitor of p38 mitogen-activated protein kinase (p38MAPK), inhibited keratinocyte migration induced by A3G756. Next, inhibitory studies using functionally blocking antibodies against integrin subunits show that incubation with the anti-a5 and -ß1 integrin antibodies significantly decreased the A3G756-induced cell migration. Furthermore, microspheres covered with A3G756 bound to syndecan-4, could cluster ß1 integrin and vinculin. These results suggested that cell stimulated by A3G756 may migrate on fibronectin produced by keratinocytes. For in vivo study, cutaneous wound was made with mouse and rabbit. Topical application of A3G756 to cutaneous wound accelerated reepithelialization. The A3G756-induced wound repair was blocked by treatment with SB203580. Our studies indicated that A3G756-induced early closure of the cutaneous wound required p38MAPK activation. By those data, it is indicated that the A3G756 peptide is likely a potential therapeutic reagent for chronic cutaneous ulcer.

Constitutive thrombospondin-1 overexpression contributes to the autocrine TGF-ß signaling in cultured scleroderma fibroblasts.

The extracellular matrix (ECM) glycoprotein thrombospondin-1 (TSP-1) has been reported to activate the latent complex of TGF-ß, the major effects of which in mesenchymal cells is stimulation of the synthesis of ECM. Previous reports suggested the involvement of an autocrine TGF-ß loop in the pathogenesis of scleroderma. In this study, we examined whether TSP-1 plays a role in maintaining the autocrine TGF-ß loop in scleroderma. TSP-1 expression was increased in scleroderma patients compared with in healthy controls in vivo and in vitro. TGF-ß blocking antibody or TGF-ß1 antisense oligonucleotide markedly reduced the up-regulated TSP-1 expression in scleroderma fibroblasts but had little effect on normal fibroblasts. The expression of TSP-1 is up-regulated in scleroderma fibroblasts possibly at the posttranscriptional level just like in normal fibroblasts stimulated with exogenous TGF-ß1. TSP-1 blocking peptide or antisense oligonucleotide had an inhibitory effect on the up-regulated a2(1) collagen and phospho-smad3 levels in scleroderma fibroblasts but had little effects on normal fibroblasts. The transient overexpression of TSP-1 up-regulated a2(1) collagen and phospho-Smad3 levels in normal fibroblasts but had no major effect on scleroderma fibroblasts. Furthermore, these effects of transiently overexpressed TSP-1, which possibly occurred via the activation of latent TGF-ß1, were abolished by the TGF-ß1 antisense oligonucleotide. These results indicate that the constitutive overexpression of TSP-1 may play an important role in autocrine TGF-ß signaling and accumulation of ECM in scleroderma fibroblasts.