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A novel small compound accelerates dermal wound healing by modifying infiltration, proliferation and migration of distinct cellular components in mice

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ABSTRACT

Background: Impaired wound healing in skin ulcer is one of the major medical issues in the aged society. Wound healing is a complex process orchestrated by a number of humoral factors and cellular components. TGF-β is known to stimulate collagen production in dermal fibroblasts while inhibiting proliferation of epidermal keratinocyte. A screening of small compounds that suppress type I collagen production in fibroblasts has identified HSc025 that antagonizes the TGF-β/Smad signal.
Objective: We examined the effects of HSc025 on dermal wound healing and elucidated the underlying mechanisms.
Methods: Effects of HSc025 on the wound closure process were evaluated in a murine full-thickness excisional wound healing model. Cell proliferation and migration were estimated using primary cultures of human keratinocytes and fibroblasts. Comprehensive analyses of gene expression profiles were performed using untreated and HSc025-treated fibroblasts.
Results: Oral HSc025 administration suppressed macrophage infiltration and accelerated wound closure as early as at day 2 after the dermal excision. Treatment of cultured keratinocytes with HSc025 counteracted the inhibitory effects of TGF-β on cell proliferation and migration. On the other hand, HSc025 stimulated migration, but not proliferation, of dermal fibroblasts independently of TGF-β. Experiments using an artificial dermis graft revealed that HSc025 stimulated migration of collagen-producing cells into the graft tissue. A cDNA microarray analysis of untreated and HSc025-treated fibroblasts identified pirin as a critical mediator accelerating fibroblast migration.
Conclusion: HSc025 accelerates wound healing by modifying infiltration, proliferation and migration of distinct cellular components, which provides a novel insight into the therapy for intractable skin ulcer.

1. Introduction

Impaired wound healing in skin ulcer is one of the major medical issues in the recent aged society [1]. The elderly population is growing faster than any other age groups in the developed countries, and the increased age is a major risk factor for insufficient wound healing due to several underlying conditions such as malnutrition, local ischemia, and low daily life activity [2]. In addition, diabetes,

Abbreviations: TGF-β, transforming growth factor-β; IFN-γ, interferon γ; IFN-β, interferon β; RT-PCR, reverse transcription-polymerase chain reaction; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; siRNA, small interfering RNA; EGFP, enhanced green fluorescent protein; H–E, hematoxylin–eosin.

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obesity, and even some medications also cause a delay in wound healing. Some patients cannot be subjected to surgical treatment because of their poor general conditions, thus the opened wounds are susceptible to ischemia, infection, fasciitis and osteomyelitis, possibly leading to life-threatening complications such as disseminated intravascular coagulation. A number of methods have been developed to treat such an intractable disease, which include the usage of anti-bacterial, debridement, irrigation, vacuum-assisted closure, oxygenation, and moist wound healing [3]. However, none of them is enough for dealing with all of the difficult cases, and a novel treatment strategy based on the mechanisms of dermal wound healing is an eager social and medical desire.

Dermal wound healing is a complex process orchestrated by a number of humoral factors and cellular components [4,5]. Wound closure can be achieved by granulation tissue formation in the dermis and re-epithelialization in the epidermis, where fibroblasts and keratinocytes play critical roles, respectively. Fibroblasts are responsible for initiating angiogenesis, epithelialization and collagen formation, and differentiate into myofibroblasts that cause tissue contraction [5].

Among a number of growth factors that orchestrate the complex sequence of cell migration, division, differentiation, and protein expression, transforming growth factor-β (TGF-β) and its intracellular mediators, Smad proteins, have been implicated in both physiological wound healing and pathological fibrosis. In normal wound healing, TGF-β produced by platelets, macrophages and lymphocytes recruits inflammatory cells, stimulates angiogenesis, and up-regulates collagen synthesis [4]. However, despite the initial prediction that the blockade of the TGF-β signal may suppress wound healing by inhibiting collagen production in the granulation tissue, a study using Smad3-null mice has clearly revealed accelerated wound healing compared with wild type animals [6]. These results therefore indicate that the cellular and molecular mechanisms are similar, but not identical, between physiological wound healing and pathological fibrosis. For example, our recent study has shown differential contribution of dermal resident and bone marrow-derived cells to collagen production during wound healing and dermal fibrosis in mice [7].

We have been studying growth factors and cytokines that antagonize the TGF-β/Smad signal as well as their implication in the treatment of organ fibrosis [8]. Among those factors, interferon γ (IFN γ) is well known to suppress progression of organ fibrosis. We have identified YB-1 as a downstream effector of IFN γ to repress transcription of human type I α2 collagen gene (*COL1A2*) [9]. Nuclear translocation of YB-1 by IFN γ antagonized the TGF-β/Smad3 signal in regulating *COL1A2* transcription *in vitro* [10], and adenovirus-mediated overexpression of YB-1 driven by the enhancer/promoter regions of murine counterpart gene (*Col1a2*) suppressed progression of liver fibrosis and enhanced the anti-fibrotic effect of IFN γ *in vivo* [11]. More recently, we have demonstrated that a novel small compound HSc025 stimulates nuclear translocation of YB-1 and ameliorates experimental fibrosis in several organs including skin, lung and liver [12,13].

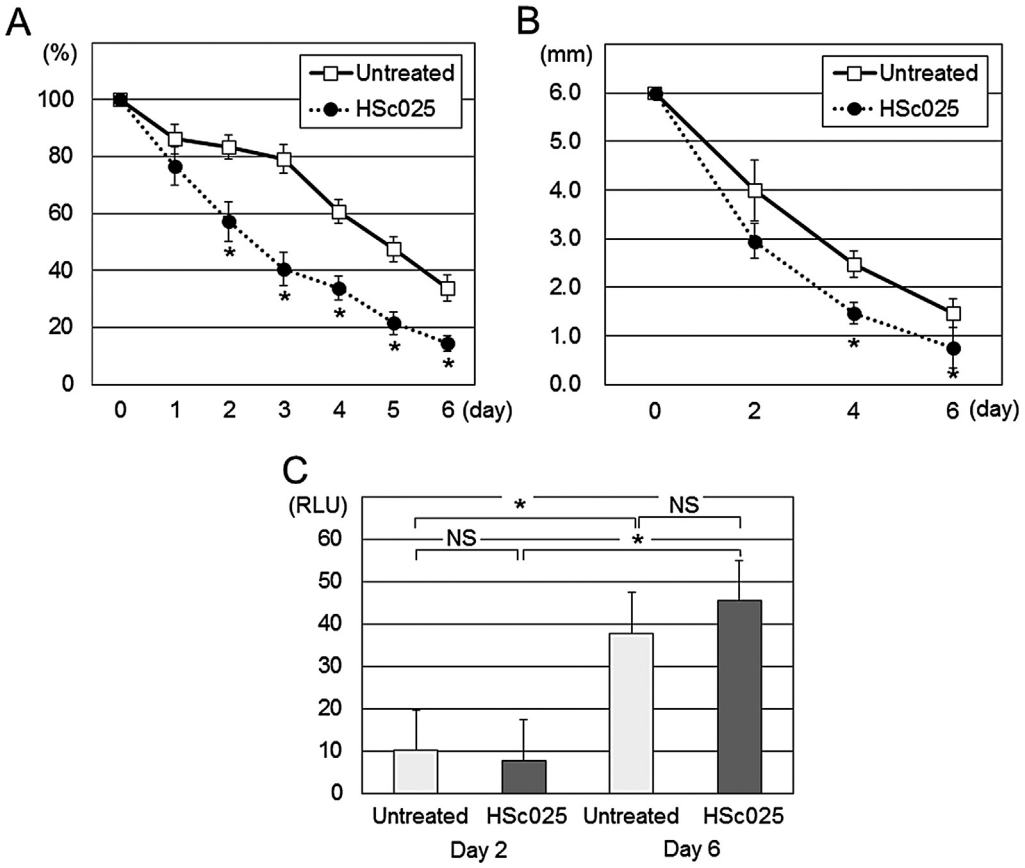

Fig. 1. Wound closure and activation of *Col1a2* promoter after a full-thickness dermal excision. Female COL/LUC mice (20 to 28 weeks of age) underwent a full-thickness 6 mm excisional wounding. They were either untreated or treated with daily oral administration of 75 mg/kg of HSc025. The wound closure was monitored everyday by measuring the area of opened wounds (A). The wound tissues were taken at day 2, 4 and 6, and subjected to H–E staining or luciferase assays to measure the distance between the both sides of wound edges (B) and *Col1a2* promoter activity (C), respectively. Luciferase activity was normalized against the protein concentration of tissue homogenates. The values are expressed as means ± SE from eight wounds in each group. An *asterisk* indicates that the difference between the groups is statistically significant. *RLU*, relative luminescence units; *NS*, not significant.