Exogenous Smad3 Accelerates Wound Healing in a Rabbit Dermal Ulcer Model

Koji Sumiyoshi,*† Atsuhito Nakao,*‡ Yasuhiro Setoguchi,† Ko Okumura,* and Hideoki Ogawa*†

*Atopy (Allergy) Research Center, Juntendo University School of Medicine, Tokyo, Japan; †Department of Dermatology, Juntendo University School of Medicine, Tokyo, Japan; ‡Department of Respiratory Medicine, Juntendo University School of Medicine, Tokyo, Japan; §Department of Immunology, Faculty of Medicine, University of Yamanashi, Yamanashi, Japan

Exogenous administration of transforming growth factor-β (TGF-β) improves wound healing by affecting cellular and molecular events involved in tissue repair. But mice with a deficiency of a key TGF-β signaling intermediate, Smad3, paradoxically showed accelerated cutaneous wound healing, suggesting that endogenous Smad3 had inhibitory effect on cutaneous wound healing. Here we investigated the effect of exogenous expression of Smad3 in dermal fibroblasts on cutaneous wound healing. Subcutaneous injection of adenovirus-containing Smad3 complementary DNA (AdCMV-Smad3) targeting mainly dermal fibroblasts accelerated tissue repair following full-thickness dermal round wounds in rabbit ear as judged by the size of granulation tissue area, number of capillaries, and re-epithelialization rate of the wounds. Expressions of α-smooth muscle actin (α-SMA), vascular endothelial growth factor (VEGF), and fibroblast growth factor receptor were upregulated in the wounded area injected with AdCMV-Smad3. Consistent with the in vivo findings, overexpression of Smad3 induced α-SMA, VEGF, and TGF-β1 expression and augmented chemotactic response in cultured dermal fibroblasts. Therefore, exogenous administration of Smad3 targeting dermal fibroblasts accelerated tissue repair in a rabbit dermal ulcer model by affecting fibroblast responses associated with wound healing. The results suggest that Smad3, when over-expressed in dermal fibroblasts, can promote wound healing.

Key words: adenovector/fibroblast/Smad7


The wound healing is a multi-step complex process consisting of inflammation, granulation tissue formation, angiogenesis, re-epithelialization, and wound contraction (Singer and Clark, 1999). When a full-thickness dermal wound is made and filled by a fibrin clot, inflammatory cells first migrate into the plasma clot and release local growth factors, such as transforming growth factor-β (TGF-β), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF), which stimulate fibroblasts from the adjacent intact dermis to migrate to the wounded site. The migrating fibroblasts, along with newly formed vessels, fill the wound, which results in the formation of granulation tissue. Activated fibroblasts differentiate into myofibroblasts, a subpopulation of specialized fibroblasts that express α-smooth muscle actin (α-SMA), which elaborate collagen and other matrix components and which eventually contract the newly formed connective tissue to bring together the edges of the wound, followed by re-epithelialization and wound closure.

TGF-β is a multi-functional cytokine involved in cellular proliferation, survival, differentiation, migration, and extracellular matrix production (Massague, 1990). Numerous evidence suggested that exogenous administration of TGF-β into the skin accelerated wound healing, either directly or indirectly, by stimulating recruitment of inflammatory cells, the production of extracellular matrix production, the formation of new blood vessels, and wound contraction (Sporn et al, 1983; Roberts, 1995). For instance, the injection of TGF-β directly to the wound at the time of wounding increased the healing rate accompanied by an increased influx of mononuclear cells and fibroblasts and by marked increases in collagen deposition at the site of application of TGF-β in rats (Mustoe et al, 1987).

Recent investigation has revealed that the action of TGF-β is mediated mainly by the Smad family of proteins (Heldin et al, 1997; Attisano and Warana, 2000; Massague, 2000). Activated TGF-β receptors phosphorylate the cytosolic receptor-activated Smad2 and Smad3, which form heteromeric complexes with Smad4, and enter the nucleus, bind to DNA, and regulate gene transcription in cooperation with DNA binding cofactors. Inhibitory Smads, Smad6 and Smad7, block TGF-β signal transduction, in part, by preventing the interaction of Smad2/3 with the activated TGF-β type I receptor.

In contrast to the prediction based on therapeutic effect of ectopic TGF-β on wound healing, Smad3-deficient mice showed accelerated wound healing associated with reduction of the influx of inflammatory cells and increased re-epithelialization (Ashcroft et al, 1999), suggesting that

Abbreviations: α-SMA, α-smooth muscle actin; cDNA, complementary DNA; FGF, fibroblast growth factor; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β; VEGF, vascular endothelial growth factor

Copyright © 2004 by The Society for Investigative Dermatology, Inc.
endogenous Smad3 functioned inhibitory for wound healing. In this study, we investigated the effect of exogenous expression of Smad3 in dermal fibroblasts on cutaneous wound healing using a rabbit dermal ulcer model.

Results

Adenovector-mediated in vivo gene transfer to the skin To determine whether exogenous Smad3 regulated wound healing, we examined the effect of exogenous Smad3 or Smad7 for comparison on tissue repair in a rabbit dermal ulcer model by using adenoviral-mediated skin gene delivery. We have previously demonstrated the feasibility of in vivo gene transfer to the skin mediated by replication-deficient adenoviral vectors (Setoguchi et al., 1994b).

Consistent with the previous report, subcutaneous injection of the adenovirus-containing lacZ cDNA (AdCMV-lacZ) into the rabbit ear showed significant expression of exogenous lacZ in the skin as judged by in situ β-galactosidase staining 2 days after the injection (Fig 1A). In higher magnification of the skin, the staining was observed largely in cells with spindle morphology (fibroblasts) in the rabbit dermis (Fig 1B).

Immunohistochemical studies with anti-Smad3 or anti-Smad7 antibody showed that the expression of Smad3 or Smad7 was detected on day 8 post-wounding after subcutaneous injection of AdCMV-Smad3 or AdCMV-Smad7 (Fig 2). It should be noted that the antibodies used for immunohistochemical detection of Smad3 or Smad7 failed to detect endogenous Smad3 or Smad7, but detected only overexpressed Smad3 or Smad7 after adenovirus infection in the tissues.

Exogenous Smad3, but not Smad7, accelerates cutaneous wound healing in rabbits We then examined the effect of in vivo gene transfer and expression of Smad3 or Smad7 on tissue repair after full-thickness wounding on rabbit ear. The amount of granulation tissue area, number of capillary lumens in the granulation tissue, and degree of re-epithelialization 8 days after the wounding were significantly enhanced in the wounded skin injected with AdCMV-Smad3 when compared with those in the wounded skin injected with AdCMV-lacZ (Fig 3). Interestingly, the injection of AdCMV-Smad3 showed a level of the wound healing equivalent to the application of total 1.5 μg of TGF-β1 (+AdCMV-lacZ) into the wounded skin. In contrast, the number of capillary lumens in the granulation tissue and degree of re-epithelialization 8 days after the incisional wounding were significantly reduced in the wounded skin injected with AdCMV-Smad7 when compared with those in the wounded skin injected with AdCMV-lacZ (Fig 3).

Because histological evaluation revealed that the injection of AdCMV-Smad3 accelerated tissue repair in a rabbit ulcer model, we next examined the effect of exogenous Smad3 on cellular and molecular events involved in wound healing. As shown in Fig 4, the number of α-smooth muscle (α-SMA) positive cells (myofibroblasts) and expression of VEGF and FGF receptors were strongly enhanced in the wounded skin injected with AdCMV-Smad3 when compared with the wounded skin injected with AdCMV-lacZ or AdCMV-Smad7. Thus, accelerated healing of dermal ulcer in a rabbit ear injected with AdCMV-Smad3 could be attributed, at least in part, to increased number of myofibroblast and increased expression of VEGF and FGF receptor in the skin.

Overexpression of Smad3 induces α-SMA, VEGF, and TGF-β1 expression and increases chemotactic response in human dermal fibroblasts in vitro Because exogenous administration of Smad3 affected granulation tissue formation, angiogenesis, and re-epithelialization in vivo, we examined the effect of Smad3 overexpression on several functions of dermal fibroblasts involved in wound healing.

Human dermal fibroblasts were infected with AdCMV-lacZ, AdCMV-Smad3, and AdCMV-Smad7 as previously described (Sumiyoshi et al., 2003) and evaluated for α-SMA expression, VEGF and TGF-β1 expression, and fibroblast chemotaxis (Figs 5–7). We found that overexpression of Smad3, but not lacZ or Smad7, strongly induced α-SMA expression as shown by western blotting and immunofluorescence studies (Fig 5). Overexpression of Smad3 also induced VEGF production (Fig 6A) and TGF-β1 expression (Fig 6B). In addition, fibroblast chemotaxis was augmented in human dermal fibroblasts overexpressing Smad3 when compared with the cells overexpressing lacZ or Smad7 (Fig 7). These results suggested that overexpression of Smad3 was sufficient to express α-SMA, VEGF, and TGF-β1 and to induce chemotactic response in dermal fibroblasts.
In this study, we showed that subcutaneous injection of adenovirus-containing Smad3 cDNA into the rabbit ear accelerated tissue repair following full-thickness dermal wounds based on improvement of histological and cellular/molecular parameters (Figs 3 and 4). Because subcutaneous injection of adenoviruses-containing Smads appeared to target mainly dermal fibroblasts in our system (Figs 1 and 2, Setoguchi et al., 1994b), the accelerated wound healing by AdCMV-Smad3 could be, at least in part, attributed to the effect of exogenous Smad3 on several fibroblast functions involved in wound healing; that is, myofibroblast differentiation (α-SMA expression), VEGF and FGF receptors, and TGF-β1 expression, and chemotactic response as shown in Figs 5–7.

Ashcroft et al. (1999) reported that Smad3-deficient mice showed accelerated cutaneous wound healing, suggesting that endogenous Smad3 had inhibitory effect on cutaneous wound healing. They suggested that downregulation of inflammation and acceleration of re-epithelialization of keratinocytes might contribute to the accelerated wound healing in Smad3-deficient mice. In contrast, we found that exogenous Smad3 resulted in increased granulation tissue formation and re-epithelialization (Fig 3). In Smad3-deficient mice, not only fibroblasts but also keratinocytes and inflammatory cells are Smad3-deficient and they observed net results of Smad3 deficiency in cutaneous wound healing. In our study, however, main target cells were dermal fibroblasts (Figs 1 and 2). Therefore, we think that this study emphasizes the activity of TGF-β1/Smad3 on dermal fibroblast responses in the process of wound healing and the difference of target cells may explain different results in these studies. Alternatively, endogenous deficiency of Smad3 throughout the development may affect the cell function that is not attributed to the molecule itself or even alter the function of cells that do not express the molecule. This may also explain the different results in these studies. In addition, it should be noted that re-epithelialization in the whole wounded areas is usually complete between days 15 and 20 post-wounding in this dermal ulcer model in the rabbit ear and this study evaluate early phase of cutaneous wound healing regarding re-epithelialization.

Previous studies reported that dermal fibroblasts expressed α-SMA, VEGF, and FGF receptors in response to TGF-β (Kikuchi et al., 1992; Desmouliere et al., 1993; Pertovaara et al., 1994). Because in vivo expression of α-SMA, VEGF, and FGF receptors after AdCMV-Smad3 injection appeared to be confined to the interstitial fibroblast-like cells (Fig 4) and overexpression of Smad3 induced these molecules in human dermal fibroblasts.
in vitro (Figs 5 and 6), it is likely that, although TGF-β activates multiple intracellular signaling pathways (Massague, 2000), “Smad3” is sufficient for mediating TGF-β-induced expression of α-SMA, VEGF, and FGF receptors in dermal fibroblasts both in vitro and in vivo.

TGF-β is a potent chemoattractant for human dermal fibroblasts (Postlethwaite et al, 1987). Our findings that overexpression of Smad3 in human dermal fibroblasts enhanced chemotaxis (Fig 7) and increased granulation tissue formation after AdCMV-Smad3 injection (Fig 3) suggested that Smad3 mediated fibroblast chemotaxis both in vitro and in vivo. It remains unclear, however, whether Smad3, either directly or indirectly, stimulates fibroblast chemotaxis in vivo.

We speculate that exogenous expression of Smad3 may have some advantages over that of TGF-β for treatment of cutaneous wounds. First, because we found that the effect of AdCMV-Smad3 on cutaneous wound healing was comparable with the effect of 1.5 μg exogenous TGF-β1 (Figs 3 and 4), exogenous expression of Smad3 may be useful for treatment of cutaneous wounds without unfavorable systemic effect of TGF-β toward other organs or systems such as tissue fibrosis and immune suppression. Secondly, recent evidence suggests that chronic wounds become unresponsive to growth factors including PDGF and TGF-β (Hasan et al, 1997; Agren et al, 1999), resulting in delayed or incomplete wound healing. For instance, Kim et al (2003) reported that fibroblasts from chronic wounds showed decreased TGF-β type II receptor expression with their lack of response to TGF-β. Thus, direct activation of intracellular TGF-β signaling by overexpression of Smad3 may be a useful way for treatment of chronic cutaneous wounds even if dermal fibroblasts in wounded areas have altered TGF-β receptor expression and lack of TGF-β response.

In summary, we showed that exogenous overexpression of Smad3 in dermal fibroblasts promoted cutaneous wound healing. Our findings suggest that modulation of Smad3 expression in dermal fibroblasts may have therapeutic potential for the treatment of cutaneous wounds.

Materials and Methods

Adenovirus vector construction and virus purification The recombinant E1-deleted adenoviral vectors carrying mouse Smad3, Smad7, or lacZ complementary DNA (cDNA) under cytomegalovirus promoters, AdCMV-Smad3, AdCMV-Smad7, or AdCMV-lacZ, were generated, purified, and transfected as previously described (Setoguchi et al, 1994a; Fujii et al, 1999).

In situ β-galactosidase staining In situ β-galactosidase staining was performed using in situ β-galactosidase staining kit (STRATEGENE) according to the manufacture’s instruction.

The rabbit ear dermal ulcer model Wounding design and sample preparation were performed according to the previously reported method (Tsuboi et al, 1995). Female white rabbits, 2.5–3.0 kg (Shiraishi Laboratory Animals, Tokyo, Japan) were anesthetized

Figure 3 Acceleration of cutaneous wound healing by exogenous Smad3, but not Smad7. AdCMV-lacZ, AdCMV-Smad3, or AdCMV-Smad7 was subcutaneously injected into rabbit skin. Two days after the injections, full-thickness dermal round wounds were made in the injected area with or without application of TGF-β1 as indicated. Eight days after the wounding, skin sections were microscopically evaluated by HE staining (A) and by quantitative analysis of granulation tissue area (B), the number of capillaries (C), and re-epithelialization rate (D) as described in the Materials and Methods. Please note that injection of AdCMV-Smad3 enhanced granulation tissue area (B), the number of capillaries (C), and re-epithelialization rate as compared with injection of AdCMV-lacZ. The arrow in figure A lacZ picture indicates the wounded area in rabbit skin.
with xylazine hydrochloride solution (10 mg per kg) (Bayer Co., Leverkusen, Germany) and ketamine hydrochloride solution (30 mg per kg) (Sankyo Co., Tokyo, Japan). After sterilization with iodine and alcohol, $5 \times 10^8$ plaque-forming units (pfu) of AdCMV-lacZ, AdCMV-Smad3, or AdCMV-Smad7 were injected subcutaneously in the inner side of the rabbit ear. The doses and timing of the adenovirus injection were determined according to our previous study (Setoguchi et al., 1994b). Forty-eight hours after the injections, four full-thickness round wounds were prepared on the injected area using a calibrated 6 mm trephine (Acu Punch, Acuderm Inc., Lauderdale, Florida) under sterile conditions (day 1). The perichondrium was kept undamaged. The wounds were covered with a sterilized transparent dressing (Tegaderm, 3M, Tokyo, Japan), and 20 µL PBS or 20 µL recombinant human TGF-β1 (R&D, Minnesota) solution (25 ng per µL: 500 ng per one ulcer) was applied to each wound using a syringe once every other day until the 5th day (on day 1, 3, and 5; total 1.5 µg per one ulcer). The ear was bandaged and kept covered throughout the experiment. The rabbits were killed on the 8th day after the wounding by intravenous administration of pentobarbital solution. All animal experiments were performed according to the approved manual of the Institutional Review Board of Juntendo University.

**Histological evaluation** After overnight fixation, the tissue was trimmed and cut through at the widest margin. The tissue was embedded in paraffin and sectioned in 5 µm increments. The sections were made perpendicular to the anterior–posterior axes and perpendicular to the surface of the wound. Three sections were placed on a slide, and stained with hematoxylin and eosin. Of the three sections on any one slide, the section with the widest original wound margin was used for assessment. The parameters measured were degree of granulation tissue area, number of capillaries, and re-epithelialization as previously described (Tsuboi et al., 1995). Each of the parameters was graded numerically as described below.

**Granulation tissue area** The amount of granulation tissue was quantified by measuring the area of granulation tissue (mm²) in the section perpendicular to the surface of the wound. Granulation tissue was traced by a computerized morphometric analysis (KS-400, Carl Zeiss Inc., Gottingen, Germany).

---

**Figure 4**

**Induction of α-SMA, VEGF, and FGF receptor expression by exogenous Smad3 in wounded skin.** AdCMV-lacZ, AdCMV-Smad3, or AdCMV-Smad7 was subcutaneously injected into rabbit skin. Two days after the injections, full-thickness dermal round wounds were made in the injected area with or without application of TGF-β1 as indicated. Eight days after the wounding, skin sections were immunohistochemically stained with antibodies specific for α-SMA (A), VEGF (B), and FGF receptor (C). Brown color indicates the positive staining. Please note that injection of AdCMV-Smad3 enhanced α-SMA (A), VEGF (B), and FGF receptor (C) expression (brown color) in the wounded skin.
Number of capillaries The number of capillary lumens in the granulation tissue was counted in the complete wound cross-section at \( \times 100 \) magnification.

Re-epithelialization The degree of re-epithelialization was measured by a computerized morphometric analysis (KS-400) and was given a value by percentage; 0% was equivalent to no closure and 100% was equivalent to complete wound closure.

Immunohistochemistry Skin paraffin sections were deparaffinized and then preincubated with normal mouse or goat serum (1:20) for 20 min. The slides were incubated overnight at 4°C with mouse monoclonal antibody against vascular endothelial growth factor (VEGF) (05-443, Upstate Biotechnology, Lake Placid, New York 1:50) or mouse monoclonal antibody against FGF receptor (MAB125, CHEMICON, Temecula, California 1:150), goat polyclonal antibody against Smad3 (sc-6202, Santa Cruz Biotechnology, Inc., Santa Cruz, California 1:100), mouse monoclonal antibody against \( \alpha \)-SMA (U7033, DAKO EPOS, 1:1), or goat polyclonal antibody against Smad7 (sc-7004, Santa Cruz Biotechnology, Inc., 1:100). Sections were then incubated for 60 min at room temperature with an Envision labeled polymer reagent (K1490, DAKO, Glostrup, Denmark). To increase their sensitivity, sections were then incubated for 10 min at room temperature with a Tyramide signal amplification (TSA) Biotin System (NEN life science products, Boston, Texas, 1:50). Sections were then incubated with a streptavidin (DAKO, 1:400) for 30 min. Between steps, the slides were rinsed for 10 min in PBS. All sections were lightly counterstained with hematoxylin.

Cell culture Primary human dermal fibroblasts were cultured as previously described (Lee et al, 1996). Briefly, fibroblasts were obtained from the outgrowth of infant foreskin and maintained in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal calf serum (FCS) and 100 µg per mL streptomycin. All experiments were carried out using fibroblasts in passage 4–8th. Infection of recombinant adenoviruses was performed at a multiplicity of infection (m.o.i.) of 2 \( \times 10^2 \) pfu per cell in 1 mL of serum-free DMEM for 60 min and overexpression of exogenous Smad3 or Smad7 in the cultured human dermal fibroblasts after the transfection was previously reported (Sumiyoshi et al, 2003). Dermal fibroblasts infected with the adenovirus solutions were cultured in DMEM supplemented with 10% FCS for 48 h and thereafter used for the following experiments.

Immunoblot Immunoblotting with anti-\( \alpha \)-SMA antibody (American Research Product, Belmont, Massachusetts 1:500) was performed as previously described (Nakao et al, 1997).

Immunofluorescence microscopy Dermal fibroblasts infected with the adenovirus solutions were grown on rat tail collagen type I-coated eight well culture slides (Beckton Dickinsons Labware, Bedford, Massachusetts) in the absence or presence of TGF-\( \beta \) (10 ng per mL) (R&D, Minnesota) for 24 h, then washed with PBS and fixed with 4% paraformaldehyde. Following permeabilization, slides were stained with anti-\( \alpha \)-SMA antibody (American Research Product, 1:3) diluted in PBS 90 min at room temperature. After extensive washing, slides were incubated with Alexa488-conjugated goat anti-mouse antibody (1:200) for 1 h at room temperature. After extensive washing, slides were incubated with DAPI solution (100 ng per mL) for 5 min. Fibroblasts were mounted in fluorescent mounting medium (DAKO), and images were acquired using a confocal microscopy (ECLIPSE E800, Nikon, Japan).
Cytokine ELISA The amount of VEGF in the culture supernatant of fibroblasts was determined using human VEGF ELISA kit (R&D, Minnesota).

RT-PCR Total RNA was prepared from cultures of dermal fibroblasts 72 h after the infection of adenoviruses carrying lacZ, Smad3, and Smad7, as recommended by manufacturer's instructions for Isogen solution (Nippon Gene, Japan). cDNA was synthesized from 3 μg of total RNA using first strand cDNA synthesis kit (Ready To Go) (Amersham Pharmacia, Piscataway, New Jersey). PCR amplification (95°C for 1 min, 58°C for 2 min, and 72°C for 2 min; 25 cycles) was performed in a thermal cycler (Perkin-Elmer, Wellesley, Massachusetts). The PCR products were size-fractionated by agarose gel electrophoresis using 2.0% agarose, and stained with 0.5 μg/mL ethidium bromide. As positive controls, we used expression constructs of human TGF-β1 cDNA. Primers used in this study were as follows: TGF-β1 (5′-AGATGGACACAGGCTTCCC-3′ and 3′-GTTATGCGGCTCCGTCTG-5′), and hypoxanthine phosphoribosyltransferase (HPRT) (5′-TTCTTGGTGCAGCTGCT-3′ and 3′-TTTCTACGATTCCAGCG-5′).

Data analysis Data are summarized as mean ± SD. Statistical analysis was performed using the unpaired Student t-test. p < 0.05 was considered to be significant.

We thank Drs Kazuko Yokomizo, Atsushi Furuhata, Toshinari Funaki, Yuko Kikuchi, Hiroko Ushio, Chiharu Nishiyama, Keiko Maeda, Toshio Takai, Tomoko Tokura for discussion and technical assistance, and M. Matsumoto and E. Kawasaki for secretarial assistance. This work was supported in part by the grant from the Ministry of Education, Culture, Sports, Science, and Technology, Japan and from the Ministry of Health, Labor, and Welfare, Japan.

DOI: 10.1111/j.0022-202X.2004.22730.x

Manuscript received October 7, 2003; revised March 3, 2004; accepted for publication March 8, 2004

Address correspondence to: Atsuhito Nakao, Department of Immunology, Faculty of Medicine, University of Yamanashi, 1110, Shimokato, Tamaho, Yamanashi 409-3898, Japan. Email: anakao@yamanashi.ac.jp

References


Figure 6 Induction of VEGF and TGF-β1 by overexpression of Smad3 in human dermal fibroblasts. Cultured human dermal fibroblasts infected with adenoviruses carrying lacZ, Smad3, or Smad7 cDNA were incubated with 10 ng/mL of TGF-β1 for 72 h. Then, human VEGF concentration in the culture supernatants was measured by ELISA (A) or mRNAs were taken for detection of TGF-β1 mRNA by RT-PCR (B). The relative density of the each PCR band from three separate experiments was estimated by using a one-dimensional image analyzer and was indicated as a bar graph. Data are indicated as the mean ± SD. Please note that overexpression of Smad3 in dermal fibroblasts enhanced VEGF (A) and TGF-β1 (B) expression.

Figure 7 Enhanced migration of human dermal fibroblasts by overexpression of Smad3. Cultured human dermal fibroblasts infected with adenoviruses carrying lacZ, Smad3, or Smad7 cDNA were subjected to migration assay as described in the Materials and Methods. Data are indicated as the mean ± SD of triplicate samples. *p < 0.05, significantly different from the mean value of the corresponding control response. Similar results were obtained in at least three independent experiments. Please note that overexpression of Smad3 in dermal fibroblasts enhanced their migration.

Migration assay Migration assay was performed using quantitative cell migration assay kit (ECM500, Chemicon International Inc.) according to the manufacturer's instruction.


