Stem Cell Factor Combined with Matrix Proteins Regulates the Attachment and Migration of Melanocyte Precursors of Human Hair Follicles in Vitro

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In conjunction with matrix proteins, stem cell factor (SCF) plays an important role in the migration of melanocyte precursors (MPs) derived from the mouse embryo. However, no studies have demonstrated an effect of SCF on human follicular MPs migration in vitro. In this report, first we demonstrate the immature state of the follicular MPs. Then cell attachment rate was measured by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide method. Standard 48-well chemotaxis chambers were used for a transfilter migration assay. F-actin was labeled by rhodamine-conjugated phalloidin, and then organization of the actin cytoskeleton was observed by confocal microscope. In the results, we directly show that MPs adhere more strongly to fibronectin (FN), laminin (LN) and type IV collagen (CIV) than to the negative control. SCF decreased the adhesion of MPs to FN and CIV. A chemotaxis analysis showed that FN and CIV have chemotactic effects on MPs. FN showed an obvious increase in chemotactic effects on MPs with SCF treatment comparing with the control group, but there were no significant changes in the levels of chemotaxis with CIV and LN when the cells were treated with SCF. SCF was chemotactic to MPs, and the presence of FN caused a statistically significant increase in MPs migration at various concentrations of SCF. Furthermore, we showed that SCF, in combination with FN, could induce an apparent increase in actin stress fiber formation in MPs. Our results indicate that SCF, in combination with matrix proteins and in particular with FN, regulates the movement of MPs by both altering cell attachment and increasing cell chemotaxis.

Key words melanocyte precursor; stem cell factor; matrix protein; attachment; chemotaxis

When vitiligo patients undergo repigmentation, they develop small pigmented islands that expand and coalesce to form normally pigmented skin. 1) It has been demonstrated that these islands of pigmentation are derived from melanocyte precursors (MPs) in the outer root sheath (ORS) of hair follicles.2) Although this phenomenon has been clinically well supported and MPs have been cultured successfully in vitro, little previous work has been performed to evaluate the migration capability of MPs.3–6)

When cells migrate, they synchronously attach and detach to various matrix proteins. During the repigmentation of vitiliginous skin, it is thought that MPs become activated, migrate along the basement membrane under the ORS into depigmented skin and then differentiate into mature melanocytes.7, 8) The major structural proteins of the basement membrane zone (BMZ) of both normal skin and hair follicles are type IV collagen (CIV), laminin (LN) and entactin. Fibronectin (FN) present in the BMZ located under the cells of the ORS is not a normal epidermal BMZ component. It can only be identified in the epidermal BMZ during embryonic development.8, 9) Therefore, there are some differences between epidermal melanocytes and MPs in the matrix proteins surrounding the BMZ. Previous authors have evaluated the impact of the matrix proteins FN, CIV and LN on epidermal melanocyte movement,10–12) but these data are lacking for MPs.

A previous immunohistochemical study showed that the majority of the KIT-reactive dendritic cells within the epidermis and upper follicular infundibulum co-expressed tyrosinase (TYR) and tyrosinase related protein-1 (TRP-1), which are markers of differentiated melanocytes. Within the deeper follicular ORS, the population of precursor melanocytes expresses only KIT and lacks the differentiated melanocytic phenotype.13, 14) As the exclusive ligand of KIT, stem cell factor (SCF) can increase melanocytes proliferation, differentiation, survival, chemotaxis and accumulation in vivo.15, 16) The major role of SCF in melanogenic phenomena has been thought to be targeting KIT-bearing melanoblasts or melanocytes, as evidenced by the migration of immature melanocytes from the neural crest toward the hair follicles via the epidermis.17) In vitro, FN combined with SCF alters the attachment and migration of cultured mouse neural crest cells, which are a type of melanocytes precursors.18) Thus, it is possible that SCF, combined with matrix proteins, might play an important role in the migration of MPs from the ORS into the epidermis during the repigmentation of vitiligo.

We have successfully cultured MPs of human hair follicles in vitro.5) Here, we will investigate the effects of SCF combined with matrix proteins FN, LN and CIV on the attachment and chemotaxis of MPs. In addition, the changes in the MPs cytoskeleton will also be analyzed to clarify the mechanism of how these proteins affect MPs.

MATERIALS AND METHODS

MPs Culture This study was approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University. MPs were cultured as described previously but

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with improvements to the culture medium. Normal human scalp skin (approximately 15–20 cm²) was obtained from two cadaveric donors (men aged 49 and 32 years) within 1 h of death and was immediately immersed in MCDB-153 medium (Sigma) supplemented with 1000 U/mL penicillin and 1000 µg/mL streptomycin. Before the scalp samples were collected, the two scalp donors were asked to sign informed consent forms. The epidermis and the upper 1 mm of dermis were removed with a scalp. Hair follicles in the remaining dermis were isolated using a two-step enzyme treatment. First, the tissue was cut into 0.5 cm² pieces and placed in MCDB-153 medium containing 0.5% dispase (Sigma, U.S.A.) and 5% fetal bovine serum (FBS) (Gibco, U.S.A.) for 12–16 h at 4°C, followed by immersion in the above medium with 0.5% collagenase IV (Sigma) for 1 h at 37°C. Contaminating dermal tissue was removed from individual hair follicles by exhaustive washing with 0.01 M phosphate-buffered saline (PBS), and then the free hair follicles were harvested by centrifugation at 2000 rpm with a 0.5 m rotor radius. Single-cell suspensions were obtained by treating the isolated hair follicles with 0.05% trypsin and 0.53 nM ethylenediamine tetraacetic acid (EDTA) for 5–10 min at 37°C. The dissociated cell suspensions were seeded into 6-well plates at a density of 1–2 x 10⁵ cells per well in MCDB-153 medium supplemented with 2% FBS, 10% chelated FBS, 1.66 µg/L cholera toxin (Sigma), 10 ng/mL nerve growth factor (NGF) (Peprotech, U.S.A.), 2.5 ng/mL bFGF (Peprotech), 100 nM ET-3 (Gibco), 0.05 mg/mL gentamicin (Sigma), 2.5 µg/mL fungizone (Sigma), 100 U/mL penicillin, and 100 µg/mL streptomycin. Chelated FBS was prepared by mixing 15 g of Chelex-100 (Sigma) per 500 mL of FBS for 1.5 h at 4°C with gentle stirring. The growth of fibroblasts is Ca²⁺ dependent. The low-calcium culture medium was used for reducing fibroblasts contamination. In our experiment, serum was first reacted with the divalent metal ion chelating agent Chelex-100 before use, thereby reducing the increase in Ca²⁺ concentration caused by the serum. Because some divalent cations are necessary for MPs culture, 2% non-chelated serum was added to provide essential nutrients. Geneticin (Sigma) was used to eliminate the contaminating fibroblasts. Keratinocytes were removed by the differential trypsinization method. The cells were incubated at 37°C in a humidified atmosphere of 5% CO₂, and the medium was changed twice a week. The cells were passaged at a 1:2 dilution when they became 80% confluent. At passage 3, the MPs were used for experiments. Epidermal melanocytes (MCs) were cultured as described in ref. 5.

Western Blotting, Transmission Electron Microscopy and 1-3,4-Dihydroxyphenylalanine (DOPA) Reactions
For demonstrating the immature state of the MPs, we performed Western blotting, transmission electron microscopy and DOPA staining. MCs were utilized as positive controls. Western blotting was performed essentially as described previously, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. Aliquots of the cytosol fraction were removed to assay for protein content using the bicinchoninic acid Protein Assay Kit (Pierce, U.S.A.). An equal amount of protein from each samples was added to 6× sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) sample loading buffer and denatured at 95°C for 5 min, followed by separation on 10% SDS-PAGE and transfer to Immobilon-P membrane (Millipore; Bedford, MA, U.S.A.) at 400 mA for 2 h. The membranes were washed, and nonspecific binding sites were blocked with 3% bovine serum albumin (BSA) (Sigma, U.S.A.) in Tris-buffered saline containing 0.1% Tween-20 (TBST). The membranes were incubated with primary antibodies to microphthalmia-associated transcription factor (MITF) (3F276) (Santa Cruz Biotechnology, U.S.A.), TYR (T311) (Santa Cruz Biotechnology, U.S.A.), TRP-1 (SPM456) (Santa Cruz Biotechnology, U.S.A.), tyrosinase-related protein 2 (TRP-2) (C-9) (Santa Cruz Biotechnology, U.S.A.) and GAPDH (sc-59450) (Santa Cruz Biotechnology, U.S.A.) in 2% BSA in TBST overnight at 4°C. Then, the membranes were incubated with goat anti-mouse immunoglobulin G (IgG)-horse-radish peroxidase for 60 min at room temperature. The levels of MITF, TYR, TRP-1, TRP-2 and GAPDH were detected using a chemiluminescence detection kit (Pierce, U.S.A.) following the manufacturer’s instructions. The amount of each protein present was determined by scanning the bands and quantifying the density of the bands using Quantity One Software (BIO-RAD, U.S.A.). The DOPA reaction and transmission electron microscopy were performed as described elsewhere. MPs fixed by 4% paraformaldehyde were incubated 0.1% DOPA (0.1 mol/L PBS) in 37°C for 5 h, and nuclei were stained by Nuclear Fast Red.

Immunohistochemical Staining
To analyze the expression of KIT, immunostaining with a mAb specific for CD117-APC (Caltag, U.S.A.) was performed. The streptavidin/peroxidase method was used according to the manufacturer’s instructions (DAKO, China), and DAB developer was employed for coloration. Negative controls were obtained by omitting the primary antibody.

Attachment Assay
Cell attachment assays were carried out in 96-well microtitrater plates coated with FN, LN and CIV, as previously described. FN (Sigma, dissolved in distilled water), LN (Sigma, dissolved in Tris buffer, pH 7.2) and CIV (Sigma, dissolved in 0.25% acetic acid) were diluted in MCDB-153 medium at various concentrations (0.5, 5, 10, 50 µg/mL) and 100 µL aliquots were dispensed in quadruplicate into 96-well cell culture dishes (Costar Corp., China). The cells were incubated overnight at 37°C, and uncoated sites on the wells were then blocked with 1% BSA in PBS for 3 h at 4°C as a negative control. MPs were harvested by incubation with 0.25% trypsine EDTA (0.53 nM), and the activity of the trypsin was stopped by the addition of FBS. The cells were then washed and resuspended in MCDB-153 culture media at a concentration of 2 x 10⁵ cells/mL, 100 µL (containing 2 x 10⁵ cells) of which was added to the wells. The wells were incubated for 90 min at 37°C. The assays were terminated by aspirating the loosely bound and unbound cells with two washes of PBS. The amount of cells remaining was determined using an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma, U.S.A.) assay. Briefly, 10 µL of the 5 µg/mL MTT solution was added into the wells filled with 100 µL of MCDB-153 medium and incubated at 37°C for 4 h. The reaction was terminated by the addition of 100 µL of dimethyl sulfoxide (DMSO), and the absorbance was read using an enzyme-linked immunosorbent assay plate reader (EL808; Biotech, America) at 570 nm. To calculate cell number per well, a standard curve of a known number of MPs plotted against MTT absorbance was determined for each experiment. Percent attachment was calculated as (attached cells num-
ber/20000) × 100. To evaluate the effects of SCF and matrix proteins on the attachment of MPs, the cells were incubated with SCF (Peprotech) at various concentrations (0, 10, 100, 500 ng/mL) for 24 h and then were plated into 96-well cell culture dishes coated with 10 μg/mL FN, LN and CIV. The subsequent steps were performed as described above. Three independent experiments with all variables in triplicate were performed.

Chemotaxis Assay  Standard 48-well chemotaxis chambers (Neuro Probe, U.S.A.), in which the upper and lower wells were separated by an 8-μm pore-size polycarbonate membrane, were used for a filter migration assay. Briefly, the chambers were filled with 40 μL of the chemottractants or control reagents that had been warmed to approximately 37°C and vortexed to expel dissolved gasses. The chemotactrant substances used were FN, LN and CIV (0, 10, 100, 500 ng/mL), and 2 × 10⁴ cells (including MPs and MPs incubated with 100 ng/mL SCF for 24 h) in 50 μL MCDB-153 medium were added to the upper compartment of the chemotaxis chamber. After a 4-h incubation, the upper surfaces of the filters were mechanically cleaned, fixed and stained to quantify the number of cells that migrated through the filters. The number of migrated melanocytes was counted using an image analysis system (100× magnification, four fields of vision by two independent investigators, each experiment with all variables in duplicate carried out three times). To examine the effects of SCF on the migration of MPs, increasing concentrations of SCF (0, 10, 100, 500 ng/mL) were used as chemotactrant substances (Peprotech). The 8-μm pore-size polycarbonate membranes were precoated on the bottom side with 10 μg/cm² FN, LN and CIV to evaluate the role of SCF combined with matrix proteins. The subsequent steps were performed as described above.

Immunofluorescence The cells were harvested from dishes using 0.05% trypsin-EDTA (0.53 mM) and were plated on glass coverslips coated with 10 μg/cm² fibronectin and allowed to attach and spread out for 4 h at 37°C. SCF (100 ng/mL) was added to the culture dishes for 2 h. The cells were then fixed in 4% paraformaldehyde for 10 min at room temperature and permeabilized in 0.1% Triton X-100 (Fluka Buchs, Switzerland)/2% BSA in PBS. For staining of the actin cytoskeleton, the cells were incubated for 30 min in rhodamine-conjugated phalloidin (Sigma), as recommended by the supplier. The coverslips that were covered with cells were rapidly washed three times with PBS (pH 7.4) and viewed under a confocal laser microscope (Leica Sepi).

Statistical Analyses The experiments were evaluated according to analysis of variance (ANOVA) and Student’s t-test using SPSS software 13.0. Data were expressed as the means ± S.D. The level of significance was set at p < 0.05 in all cases.

RESULTS

Culture and Identification of MPs Primary human MPs typically have a triangular or bipolar morphology similar to that reported for human melanoblasts⁴⁻⁶ (Fig. 1A). Western blotting showed that these cells expressed MITF, which suggests that the cultured cells have melanocytic origins (Fig. 1B). To demonstrate that the MPs were undifferentiated, we checked for the expression of the critical enzymes of melanin synthesis, including TYR, TRP-1 and TRP-2. We found that these cells expressed TYR, TRP-1 and TRP-2. However, the expression levels of TYR obviously lower than the levels observed in MCs. In addition, MITF and TRP-1 were expressed at slightly lower levels in the MPs than in MCs. There were not apparent differences in the levels of TRP-2 between the two types of cells (Fig. 1B). As further proof, we found that the cells are amelanotic and unreactive to DOPA (Figs. 1C, D), indicating a lack of active TYR, which is consistent with the cells containing predominantly stage I and II melanosomes, as visualized by transmission electron microscopy (Figs. 1E, F). In contrast, the positive control consisting of MCs was reactive to DOPA staining, and many stage III and IV melanosomes could be found in these cells. It is well known that MPs do not express TYR in vivo. However, our cultured cells expressed low levels of TYR. Thus, we speculate that these cultured cells have undergone a minor amount of differentiation, in contrary to MPs in vivo. Nevertheless, the cultured MPs were immature and amelanotic, similar to MPs in vivo; therefore, we proceeded to perform the following migration studies.

Our immunohistochemical analysis showed that KIT was expressed in the cytoplasm and at the cell membrane (Fig. 1G). These results suggest that SCF has an indirect regulatory effect on melanocytes. As we all know, melanin in the mature melanocytes can show the similar color as DAB developer. We set negative controls by omitting the primary antibody to prevent the possible false positive results. No pigment granules were found within the cytoplasms in the negative control group, which further demonstrate that the cultured MPs are amelanotic (Fig. 1H).

MPs Attachment In general, FN, LN and CIV can increase MPs attachment in the manner of concentrations. The MPs adhered more strongly to FN and LN than to CIV (p < 0.01). There were no obvious differences between the attachment of MPs to FN or to LN (Fig. 2). In the negative control group (0 μg/mL matrix proteins), the attachment of the MPs to 0.1% BSA-coated wells was less than 1% in all experiments.

To determine the effect of SCF on the adhesion of MPs to matrix proteins, the MPs were incubated with SCF (10, 100, 500 ng/mL) for 24 h, and then an attachment assay was performed. Negative control samples were obtained by incubating MPs with the vehicle used for dissolving SCF. As illustrated in Fig. 3, following SCF treatment, the MPs exhibited a significant decrease in attachment to FN and CIV (p < 0.01). In contrast, the MPs showed an apparent increased attachment to LN following SCF treatment (p < 0.05). The regulatory effects of SCF on MPs attachment to FN, LN and CIV occurred in a dose-dependent manner.

Chemotaxis The ability to migrate over matrix proteins has been shown to be important for many forms of cell movements, and matrix proteins have close relationships with the movements of melanocytes. CIV can significantly increase melanocyte migration. FN also has been shown to exert chemotactic effects on melanocytes. To characterize the effects of the matrix proteins FN, LN and CIV on MPs movements, we performed a chemotaxis analysis using 48-well chemotaxis chambers. FN and CIV showed apparent chemotactic effects by increasing the migration of the MPs in a dose-dependent manner compared with the negative control group (Fig. 4). No chemotactic behavior could be observed with any tested reagents that had been warmed to approximately 37°C.
Fig. 1. Culture and Identification of MPs

(A) Primary human MPs typically had bipolar morphology (100 µm —). (B) The MPs expressed MITF, TYR, TRP-1 and TRP-2. However, the expression levels of TYR were obviously lower in MPs than in MCs. In addition, MITF and TRP-1 were expressed at slightly lower levels in MPs than in MCs. (C, D) MPs are negative for DOPA, while MCs are positive (nuclei were stained by Nuclear Fast Red, 50 µm —). (E, F) The cells contained predominantly stages I and II melanosomes, as visualized by transmission electron microscopy. In contrast, numerous stages III and IV melanosomes could be observed in MCs (200 nm —). (G, H) Immunohistochemical staining showed the expression of KIT at the cell membrane and in the cytoplasm of the MPs. The negative control cells did not contain any pigment granules (SP, DAB coloration, 25 µm —).
concentration of LN. Less than 3 migrating cells could be observed occasionally in the negative control group (0 ng/mL matrix proteins). To determine whether SCF regulates the chemotaxis of matrix proteins to MPs, a chemotaxis assay was performed using MPs incubated with 100 ng/mL SCF for 24 h. FN combined with SCF treatment resulted in a significant increase in MPs chemotaxis compared with the untreated group (p < 0.01) (Fig. 4). There was no significant difference in the amount of MPs migration with CIV or LN in combination with SCF (Fig. 4). These results suggest that SCF might only improve the migration of MPs in the presence of FN.

SCF increases melanocyte chemotaxis, survival and accumulation in vivo. In particular, it acts as a chemotactic factor for the migration of melanocyte precursors into the epidermis and hair follicles. There is no significant chemotactic effect of SCF on mature melanocytes in vitro; however, it enhances melanocyte chemokinesis.21 Here, we determined the chemotactic effects of SCF on MPs and observed the regulatory effects of FN, LN and CIV on the chemotaxis induced by SCF by coating filters with matrix proteins prior to performing chemotaxis assays. We found that SCF had chemotactic effects on the MPs, with a significant increase in the number of migrating cells as its concentration increased. The presence of FN caused a statistically significant increase in the migration of MPs at various concentrations of SCF (p < 0.01) (Figs. 5A–C), whereas the MPs did not migrate in response to SCF in the presence of LN and CIV. Thus, these results suggest that FN contributes to the migration of MPs induced by SCF.

SCF Combined with FN Induced Changes in the MPs Cytoskeleton Cell motility is a dynamic process involving the synthesis, organization and breakdown of cytoskeletal elements and focal contacts. The driving force for cell migration is directed by the reorganization of the actin cytoskeleton.22 We have shown that SCF combined with FN regulates MPs migration and attachment, as described above. To further investigate the effects of SCF combined with FN on the MPs cytoskeleton, the cells were plated on substrata covered by FN and then stained with phalloidin to visualize the actin cytoskeleton. MPs attached to the uncoated coverslips did not contain identifiable stress fibers, and only the actin-rich meshwork in the perinuclear area could be observed (Fig. 6A). MPs plated on FN exhibited actin stress fibers that were sparse and thin (Fig. 6B). SCF increased the density and thickness of these stress fibers in MPs plated on FN and caused the morphology of the MPs cell bodies to become flattened (Fig. 6C).

DISCUSSION

The repigmentation of vitiligo lesional skin is a complex process. There are three types of melanocytes that supply pigment cell sources for vitiliginous lesions during repigmentation of vitiligo, including MPs from human hair follicles and the melanocytes presenting in and around the vitiliginous lesions.2,23,24 It is also known that the migration of MPs from the ORS into the depigmented epidermis is crucial for the repigmentation of vitiliginous skin. During the repigmentation
Fig. 4. The Migration of MPs to FN, LN, and CIV

FN and CIV showed chemotactic effects on the MPs compared with the untreated control (**p<0.01), while LN affect the migration of the MPs. SCF significantly increased the chemotactic effects of FN on MPs (**p<0.01) but does not regulate the effect of CIV and LN on MPs. The data represent the mean±S.D. (n=48).

of vitiligo, MPs must move into an environment containing the cytokines secreted by keratinocytes and matrix proteins of the BMZ. The movement of various cell types is stimulated or inhibited by different cytokines combined with matrix proteins. Some studies have demonstrated that matrix proteins and cytokines regulate epidermal melanocytes migration, but it is unknown whether MPs migrate in a similar manner. Epidermal melanocytes are very different from MPs in the ORS of the hair follicle. Although they are likely to be similar with respect to migration, it is necessary to study MPs movement and its regulation by cytokines and matrix proteins to clarify the mechanism of repigmentation in vitiliginous lesions.

SCF might be locally produced by keratinocytes and fibroblasts in the skin. Previous studies suggested that SCF/KIT signaling is involved in the mechanism of melanocyte migration, such as melanocyte migration from the neural crest into the epidermis, melanocyte distribution in the hair follicle during fetal development, melanocyte migration in chemotherapy-induced hair loss and increased migration of melanocytes into the epidermis caused by local SCF injection. In vitro, SCF stimulates epidermal melanocyte chemotaxis and increases the speed of melanocyte movement on FN-coated dishes, but the effect of SCF on MPs migration was unknown. Thus, our objective was to determine whether SCF and the matrix proteins FN, LN and CIV are chemotactic to MPs and whether SCF has synergistic effects with these matrix proteins.

It was particularly important to demonstrate that cultured MPs in vitro have similar biological properties as in vivo MPs for our studies. It is well known that MPs expressed TRP-1, TRP-2 and gp100 but not TYR. Thus, MPs are unable to synthesize melanin in vivo. Indeed, our cultured MPs expressed TRP-1 and TRP-2 and low levels of TYR. With regard to the expression of TYR, the cultured MPs had possibly undergone a minor amount of differentiation. Nevertheless, we further demonstrated that the cultured MPs cannot synthesize melanin, as demonstrated by a lack of DOPA staining. The MPs did not contain mature melanosomes, as observed by transmission electron microscopy. These results suggest that the cultured MPs were undifferentiated and were characterized by the same main features as in vivo MPs. Thus the cultured MPs could be used for our experiments. In addition, our immunohistochemistry analysis indicated that the MPs expressed KIT, the exclusive ligand of SCF. This result suggests that SCF possibly plays a role in the regulation of the biological behavior of MPs.

Our results demonstrate that FN and CIV can induce MPs chemotaxis, and SCF enhances the effects of FN. We further examined MPs migration induced by SCF combined with or without the addition of FN, LN and CIV. In the absence of matrix proteins, the migration of MPs induced by SCF was clearly observed. However, FN significantly increased the chemotactic effects of SCF, whereas LN and CIV did not affect MPs chemotaxis. These results show that SCF combined with FN, but not LN and CIV, had a synergistic effect on MPs migration in vitro.

It is clear that cell movement involves a delicate balance between the ability of the cell to adhere to the underlying substratum and to detach and move along the substratum. Stronger adhesion is likely to keep the cells attached at one place, thereby preventing migration. Our results show that a decrease in the attachment of MPs to FN and CIV can facilitate the migration of these cells over the matrix proteins after SCF treatment.

During vitiligo repigmentation, MPs must move to the environment adjacent to the basement membrane zone under the ORS and epidermis. The distribution of CIV and laminin is consecutive all along the follicle. Fibronectin is not a normal component of the basement membrane zone of epidermis, but it could be expressed by the basement membrane/connective tissue sheath region of the lower follicle. Thus, FN, LN and CIV are tightly adjacent to each other, with MPs localized in the deeper follicular regions, where KIT is expressed on the surface of MPs. Therefore, it is likely that the chemotaxis and attachment of MPs regulated by SCF combined with matrix proteins in vitro shares similar characteristics as MPs migration from the ORS into the epidermis, although the environment is more complicated in vivo. The decreased attachment of MPs to FN and CIV caused by SCF most likely facili-
tated the migration of MPs along the basement membrane of the ORS, and the chemotactic effects of SCF combined with FN supplied a driving force for MPs migration.

The process of cell migration consists of multiple steps involving the coordinated action of cell matrix receptors, growth factors, and intracellular signaling events that result in the assembly and disassembly of the actin cytoskeleton. In-

Fig. 5. Chemotaxis of SCF Combined with FN, CIV and LN to MPs
(A) SCF (500 ng/mL) causes obvious migration of MPs under light microscopy (Haemotoxylin staining, 100 μm —). (B) The chemotactic effects of SCF were increased after the polycarbonate membranes were coated with FN, as determined by light microscopy (Haemotoxylin staining, 100 μm —). (C) SCF was chemotactic to MPs in a dose-dependent manner. The presence of FN caused a statistically significant increase in the migration of MPs at various concentrations of SCF compared with the control group (**p < 0.01), while CIV and LN did not affect the chemotaxis of SCF to MPs. The data represent the mean ± S.D. (n=48).
tegrins serve as a bridge between the matrix proteins and the actin cytoskeleton through the attachment of the cytoplasmic tail of the β1 and β3 integrin molecules to the cytoskeleton-associated proteins talin and α-actinin. SCF has been shown to increase the speed of the movement of epidermal melanocytes over FN by inducing organization of the melanocyte cytoskeleton.43) Our results demonstrate that FN induces the formation of stress fibers, and SCF enhances this effect. The effect of SCF combined with FN on the organization of the MPs skeleton is similar to that observed with epidermal melanocytes. It is well known that cell migration is a continuous process associated with changes in the cytoskeleton. The results presented in this report suggest that SCF combined FN promotes the migration of MPs from the ORS into the epidermis by reconstruction of the MPs cytoskeleton during the repigmentation in vitiligous lesions. The mechanism of SCF possibly involves altered expression or affinity of integrins on the MPs surface, which has been shown in other reports.18)

In summary, our results indicate that SCF, in combination with matrix proteins, promotes the movement of MPs both by altering the attachment to matrix proteins and by increasing chemotaxis of the cells in vitro. The combination of SCF with matrix proteins potentially plays an important role in the migration of MPs from the ORS into the epidermis during the repigmentation of vitiliginous skin. However, this hypothesis must be further examined with more experiments in vitro.

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