The SCF/KIT Pathway Plays a Critical Role in the Control of Normal Human Melanocyte Homeostasis

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During development, the interaction of stem cell factor (SCF) with its receptor, KIT, is critical for the survival of melanocytes. Limited *in vivo* human studies have suggested a possible activating role of SCF on adult human melanocytes. In order to study the impact of this pathway on normal melanocyte homeostasis, human skin xenografts were treated with serial injections of recombinant human SCF or a KIT-inhibitory antibody (K44.2). On histologic evaluation, SCF injection increased, whereas KIT inhibition decreased the number, size, and dendricity of melanocytes. Immunohistochemical expression of melanocyte differentiation antigens, including tyrosinase-related-protein-1 and gp100/pmel17, was markedly increased by treatment with SCF, and decreased by K44.2 treatment. The number of Ki67-positive melanocytes was increased in the SCF-treated tissue, suggesting a direct proliferative effect of SCF; conversely, treatment with K44.2 resulted in melanocyte loss, which did not appear reversible with prolonged treatment. These findings demonstrate that the SCF/KIT pathway remains critical in adult human skin, and that pharmacologic modulation of this single pathway can control cutaneous melanocyte homeostasis. *Key words: HMB-45/K44.2/mast cell growth factor/xenograft. J Invest Dermatol 111:233–238, 1998*

The regulatory processes that maintain the number and activity of melanocytes within normal human skin are not well understood. Disorders of this regulatory process may result in melanocyte loss (as in graying hair, vitiligo, or idiopathic guttate hypomelanosis) or proliferation (as in melasma, melanocytic nevus, or melanoma). Melanocytes of human skin normally survive within a cellular milieu that conditions their existence. Keratinocytes, in particular, produce many factors that potentiate the survival and growth of melanocytes (Gordon *et al*, 1989; Donatien *et al*, 1993; Herlyn and Shih, 1994; Hara *et al*, 1995). One intriguing pathway for such regulatory control is the interaction of keratinocyte-derived stem cell factor (SCF, also known as mast cell growth factor, KIT ligand, and steel factor) with its receptor, KIT, present on the surface of the melanocyte. The gene for SCF is located on chromosome 12 and is variably spliced, yielding two mRNA products encoding membrane-bound proteins of 248 amino acids and 220 amino acids (Morstyn *et al*, 1994). KIT is a 145 kDa transmembrane class III tyrosine kinase receptor encoded on chromosome 4 (Yarden *et al*, 1987; Vandenbark *et al*, 1992). Upon binding to SCF, KIT dimerizes and autophosphorylates (Blume-Jensen *et al*, 1991) and activates downstream pathways that may include PI3K, PLCγ, MAP2K, Raf1, and JAK2 (Yee *et al*, 1993; Weiler *et al*, 1996).

The SCF/KIT pathway is critical for melanocytic, reproductive, and hematologic development. Homozygous, severe defects in either SCF or the KIT receptor give rise to mice that are white-coated, sterile, anemic, and often nonviable (Bernstein *et al*, 1991; Halaban and Moellmann, 1993; Morstyn *et al*, 1994). It appears that during development of these mutants cutaneous melanocytes do not survive and therefore fail to pigment hair. The crucial role of the SCF/KIT pathway for developing murine melanocytes has been further demonstrated through experiments utilizing an anti-SCF antibody, ACK2, that specifically blocks its activation of Kit (Nishikawa *et al*, 1991; Okura *et al*, 1995; Yoshida *et al*, 1996). The SCF-KIT pathway is also critical for the development of human melanocytes. Patients with piebaldism, a disorder presenting at birth with amelanotic patches on ventral and/or acral skin surfaces, but apparently lacking detectable defects in germ cells or the hematologic system, are heterozygous for *c-KIT* mutations (Giebel and Spritz, 1991; Spritz *et al*, 1992; Ezoe *et al*, 1995). These findings demonstrate that, at least at the developmental level, the SCF/KIT pathway is of critical importance to melanocyte survival.

We have demonstrated previously that SCF injection causes activation of adult human epidermal melanocytes *in vivo*, resulting in increased numbers and HMB-45 immunoreactivity of melanocytes (Grichnik *et al*, 1995). Others have subsequently confirmed these findings (Costa *et al*, 1996). Further, melanocytes *in vitro* have also been reported to proliferate in response to SCF (Funasaka *et al*, 1992; Reid *et al*, 1995). Although no specific inhibition of human melanocytes has been reported *in vivo*, KIT anti-sense oligonucleotides decrease proliferation of cultured human melanocytes *in vitro* (Spritz, 1994). These findings suggest that the SCF pathway may have a continuing influence on melanocytes, not limited to events during development.

In order to explore the potential role of the SCF/KIT pathway in the control of normal cutaneous melanocyte homeostasis, we have performed intracutaneous injections of SCF or KIT-inhibitory antibody (K44.2, Blechman *et al*, 1993, 1995) into xenografts of normal human skin. This system is advantageous because it allows for the *in vivo* study of these agents on human melanocytes, within an intact cutaneous cellular environment, but without potential associated patient risks. Further, human SCF, while activating the human KIT receptor, does not activate the murine KIT receptor (Matous *et al*, 1996) and the K44.2 is a mouse monoclonal antibody against the human KIT receptor without known activity against the murine Kit receptor. Thus possible influences from the murine humoral or murine cellular system in response to the human SCF or K44.2 antibody are minimized.
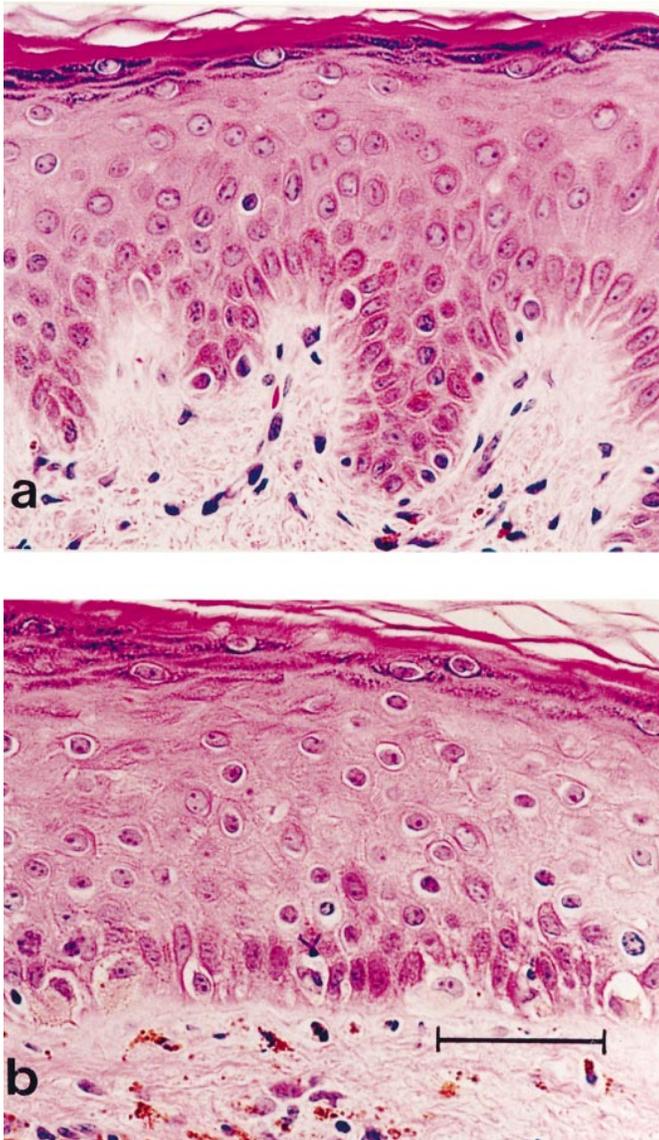
Figure 1. SCF injection results in an apparent increase in melanocyte size. Photomicrographs of hematoxylin and eosin of formalin-fixed xenografted tissue are shown. (*a*) PBS-treated tissue revealing the presence of normal-appearing melanocytes. (*b*) Two microgram SCF-injected tissue revealing enlarged melanocytes with abundant cytoplasm. *Scale bar*, 50 μm.

Using this model, in this report we demonstrate that the SCF/KIT pathway is functional in adult human skin. Pharmacologic modulation of this pathway within the cutaneous environment suggests that it plays a critical role in the control of human melanocyte homeostasis.

MATERIALS AND METHODS

Human skin xenografting   All experiments were conducted with the approval of the animal use and care committee. NIH-III mice (nude, beige, and x-linked immunodeficient) were purchased from Charles River Labs (Wilmington, MA). Human skin was obtained as residual tissue from plastic surgery procedures from five fair-skinned patients, and grossly lacked pigmentary abnormalities. The tissue was stored at 4°C in Dulbecco's minimal essential medium, supplemented with 10% fetal bovine serum, Gentamicin, and Fungizone (Life Technologies, Grand Island, NY) and grafted within 24 h of procurement. Prior to all surgical procedures, mice were anesthetized by intraperitoneal injection of 90 mg ketamine (Aveco, Fort Dodge, IA) per kg and 10 mg xylazine (Haver, Shawnee, KS) per kg. The graft was placed on a glass coverslip, and inserted dermis–down into a flank incision; the wound was sutured and dressed with polysporin ointment. After 21–35 d, the graft was exposed by excising the overlying mouse skin and removing the coverslip. The
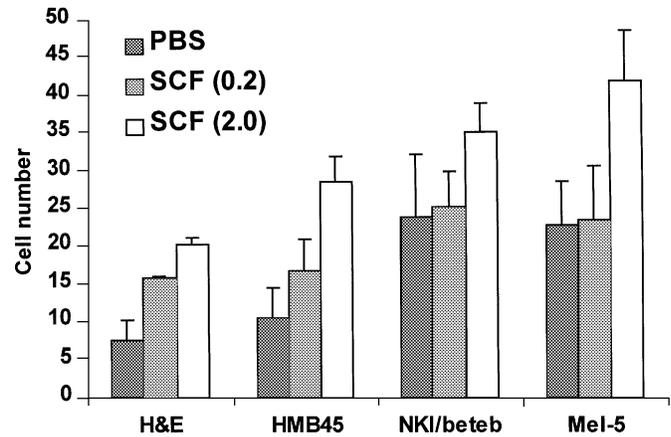
Figure 2. SCF injection results in an increase in the number of identifiable melanocytes. Human skin xenografts were injected with four once-daily injections of 0.2 μg SCF, 2.0 μg SCF, or PBS. The xenografts were harvested 2–4 h after the last injection and processed for routine hematoxylin and eosin and immunohistology with HMB45, NKI/beteb, and Mel-5. The first and second experiments included xenografts injected with PBS, 0.2 μg SCF, and 2.0 μg SCF, whereas the third experiment included xenograft-injected PBS, and two different sources of 2.0 μg SCF. The hematoxylin and eosin and immunostained specimens of each injection for all three experiments were evaluated and the data expressed as the cell numbers per 0.5 mm epidermal length (mean ± SEM, PBS, n = 3, 0.2 μg SCF, n = 2, 2.0 μg SCF, n = 4).
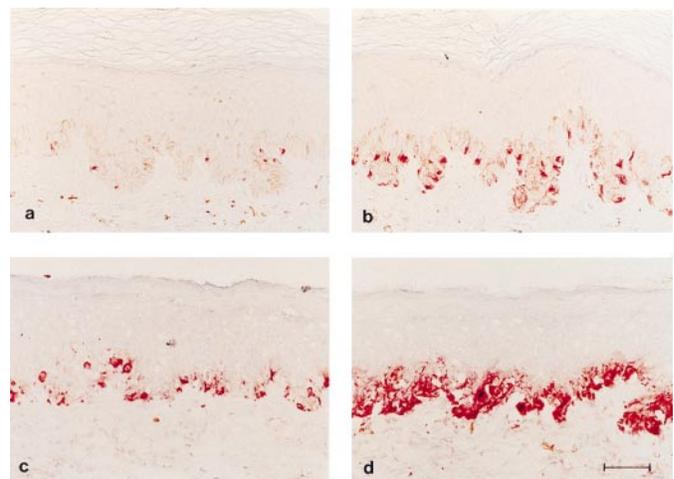
Figure 3. SCF injection results in an apparent increase in the intensity and number of melanocyte–antigen–reactive cells. The xenografted tissue in (*a*) and (*c*) was treated with PBS whereas that in (*b*) and (*d*) was treated with 2.0 μg SCF. Immunohistochemical staining with HMB-45 on formalin-fixed paraffin-embedded tissue is shown in (*a*) and (*b*). Immunohistochemical staining of frozen section with Mel-5 is demonstrated in (*c*) and (*d*). *Scale bar*, 50 μm.

grafts were allowed to mature for 6–13 wk, until the surfaces of the grafts were smooth and without scale. After treatment, the grafts and adjacent mouse skin were excised, frozen in embedding medium, or fixed in formalin, processed, and embedded in paraffin.

Injection procedure   The treatment agents or phosphate-buffered saline (PBS) (negative control) were injected intradermally through a 3/10 ml insulin syringe (Becton Dickinson, Franklin Lakes, NJ) into the xenografted human tissue. Injection volumes ranged from 25 to 100 μl. Six experiments are reported here.

In the first and second experiment, 0.2 μg and 2.0 μg of SCF were compared with diluent. In the third experiment, two different 2.0 μg SCF preparations [from two different sources, Peprotech and Sigma (St. Louis, MO)] were compared with diluent. In the first experiment, 0.2 μg and 2.0 μg of SCF (Peprotech, Rocky Hill, NJ) were dissolved in 100 μl of PBS. The volume was reduced to 50 μl of PBS for the second experiment. In the third experiment, the two 2.0 μg SCF preparations were diluted in 50 μl PBS. In the first three

Table I. SCF-treated xenografts have an increased number of melanocytes in the cell cycle compared with control PBS-injected tissue

Experiment no.	PBS	SCF (2.0 μg)
1	0.3 (1/324)[a]	3.7 (17/457)
2	0.0 (0/225)	1.8 (8/447)
3	0.6 (5/867)	3.2 (30/950)
		2.0 (17/864)[b]

[a]Percentage of melanocytes colabeling with Ki67 and Mel-5 (no. of double positive cells/total Mel-5 positive cells).

[b]Two different 2.0 μg SCF preparations were tested in experiment 3.
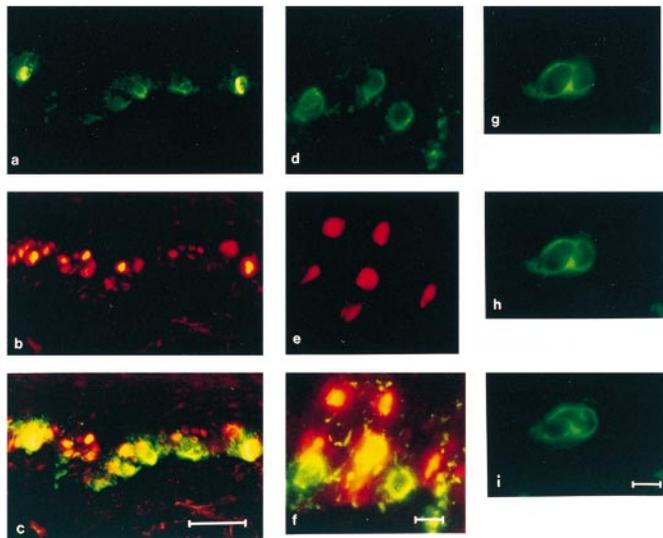
Figure 4. SCF treatment of xenografted tissue results in increased melanocytes in the cell cycle. (a–f) Double-immunolabeled, SCF-injected tissue. Mel-5 is represented in green and Ki67 is represented in red. Co-localizing cells are yellow. Parts (a)–(c) are of a 40× field demonstrating two cycling melanocytes. (d–f) A 100× view of a second field demonstrating a single positive melanocyte within a field of negative melanocytes and cycling keratinocytes. (g–i) Different focal planes through an unusual melanocyte in the SCF injected tissue revealing its large size and convoluted nucleus. This cell was not Ki67 positive. Scale bars: (a–c) 50 μm; (d–i) 100 μm.

experiments, the grafts were injected with SCF or PBS daily for 4 d and harvested 2–4 h after the fourth injection.

Murine anti-human-KIT receptor antibody (K44.2, IgG_1, Sigma) was injected at 100 μg in a 50 μl volume for experiments 4 and 5, and at 50 μg in 25 μl for experiment 6. In experiment 5, SCF (2.5 μg in 50 μl) and nonimmune murine IgG (100 μg in 50 μl, Sigma) were also used in addition to the PBS diluent control. The xenografted tissue was injected daily for 3 d and harvested on day 4 in experiment 4, injected for 4 d and harvested on day 5 in experiment 5, and injected for 8 d and harvested on days 0, 9, and 19 for experiment 6.

Tissue processing and histologic studies Standard tissue processing and immunohistochemical techniques were used as previously described (Grichnik et al, 1996). For the frozen section immunohistology, monoclonal antibodies included NKI/beteb (IgG_{2b}, 1:50, Monosan, Uden, Netherlands), Mel-5 (clone Ta99, IgG_{2a}, 1:50, Signet, Dedham, MA), anti-KIT (IgG_{2b}, 1:50, Chemicon, Temecula, CA), and isotype-specific secondary antibodies (goat anti-mouse IgG_{2a}, and anti-IgG_{2b}, Sigma). The use of the isotype-specific antibodies was necessary to reduce background staining from endogenous murine IgG_1 and the injected K44.2 murine IgG_1 antibody. The paraffin sections were reacted with a peroxidase-conjugated HMB45 monoclonal antibody (prediluted, DAKO, Carpinteria, CA). Histologic evaluation was conducted jointly by C.S., our dermatopathologist, and J.G. Cells with clearly defined nuclei surrounded by a rim of immunostain (or a rim of clear cytoplasm on the hematoxylin and eosin stained sections) were individually counted. Assessments of antigen expression were based on a per cell comparison with control-treated tissues processed in an identical manner. Double immunofluorescence was also employed as previously described (Grichnik et al, 1996). Antibodies utilized for double immunofluorescence were Mel-5 (1:25), Ki67 (rabbit polyclonal 1:25, DAKO),

and anti-KIT (1:50). FITC-labeled anti-IgG_{2a} and Cy3-labeled anti-rabbit IgG (Caltag, South San Francisco, CA) were used as secondaries. Staining for nuclear-nicked DNA ends to assess apoptosis was conducted on paraffin sections utilizing the ApopTag kit (Oncor, Gaithersburg, MD) in accordance with the manufacturer's instructions.

RESULTS

SCF stimulation results in increased size, number, and differentiation antigen expression of melanocytes No gross change in pigmentation as a result of the four once-daily injections of SCF was noted. Histologically, there was prominent enlargement and an apparent increase in the number of melanocytes in the 2.0 μg injected tissue (**Figs 1**, **2**), compared with PBS-injected control skin. We chose to further evaluate this phenomenon immunohistochemically. Because melanocytic antigen expression is dynamic, with the amount of particular antigen expressed being dependent on the local conditioning of each cell, we chose three different antibodies, HMB45, NKI/beteb, and Mel-5. HMB 45 identifies a gp100/pmel17 epitope that is prominent on "stimulated" melanocytes and melanoma (Smoller et al, 1989, 1991; Adema et al, 1993). NKI/beteb identifies a gp100/pmel17 epitope present in normal adult melanocytes (Adema et al, 1993). Mel-5 identifies tyrosinase related protein 1 (TRP-1)/gp75 (Thomson et al, 1985; Bouchard et al, 1994), which is encoded on a different gene from gp100/pmel17 and therefore coordinate regulation is not necessarily to be expected. Immunohistochemical staining of paraffin sections revealed an increased number of HMB-45 immunoreactive cells and a general increase in intensity of HMB-45 staining per cell in the 2.0 μg injected tissue compared with PBS-injected controls (**Figs 2**, **3a**, **b**). Grafts injected at the 0.2 μg level had an intermediate level of change.

Immunohistochemical studies of the frozen tissues revealed a similar pattern. Expression of TRP-1, as detected with Mel-5 immunoreactivity, was strikingly increased in the SCF-injected specimens compared with the PBS-injected control tissue (**Fig 3c**, **d**). The increase in antigen intensity was prominent for the melanocyte antigens identified by Mel-5, NKI/beteb, and HMB-45 but there also appeared to be a general increase in the number of identifiable melanocytes (**Fig 2**). In some specimens, TRP-1 staining was confluent, and the number of reactive cells reported in **Fig 2** is therefore considered a minimum value, because of the difficulty of conclusively identifying individual cells. NKI/beteb staining also showed an increase in identifiable cells with SCF treatment. KIT antigenic levels were relatively low in the xenografted tissues in these three experiments, not allowing for an accurate quantitative assessment of cell numbers; however, further diminution of this signal was noted in the xenografts that had been treated with SCF.

SCF injection increases the percentage of melanocytes in the cell cycle In order to determine whether SCF actually increased melanocyte numbers, rather than making pre-existent melanocytes more apparent due to increased cell size and immunoreactivity, the percentage of melanocytes in the cell cycle was determined immunohistochemically (**Table I**). By double immunofluorescence, cycling cells were identified by Ki67, whereas melanocytes were identified with Mel-5 (**Fig 4**). The fraction of cycling melanocytes in the SCF-treated tissue was several-fold greater than in the PBS-injected control tissue (**Table I**). Occasional melanocytes in the injected tissue were large, with unusual-nuclear morphology (**Fig 4g–i**).

Inhibition of KIT results in decreased melanocytic antigen expression, size, and numbers of melanocytes The K44.2-treated tissue in the fourth experiment revealed decreased size and number of melanocytes and markedly decreased TRP-1 staining. This was repeated in a fifth experiment in which SCF was included in addition to controls injected with PBS or nonimmune murine IgG (**Figs 5**, **6**). Hematoxylin and eosin stained sections revealed the anticipated melanocytic enlargement and increased melanocyte numbers in the SCF-treated tissue (**Figs 5a**, **6**). This was in marked contrast to the K44.2-treated tissue (**Figs 5c**, **6**), in which only scattered, small melanocytes could be identified. Immunohistochemical staining with
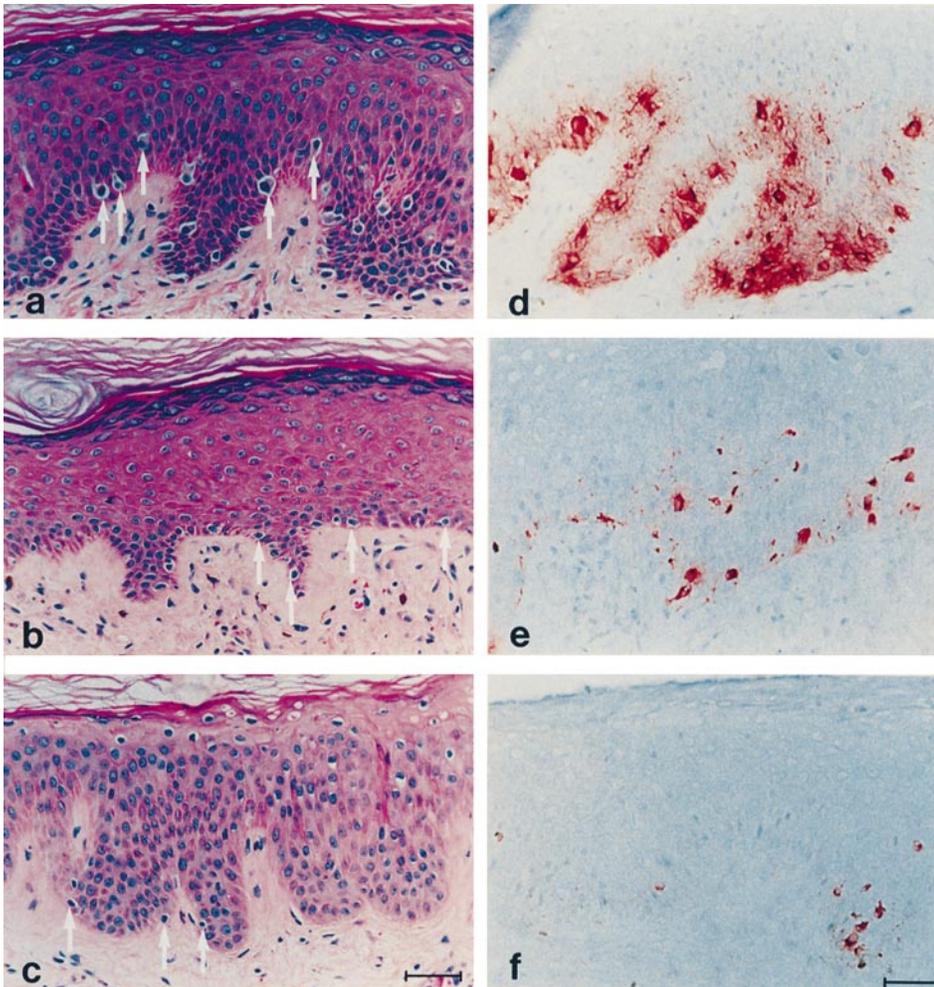
Figure 5. KIT inhibitory antibody treatment results in decreased differentiation antigen expression and loss of melanocytes. Normal human skin xenografts were injected daily for 4 d with 50 μl of solution containing 2.5 μg SCF (*a* and *d*), diluent PBS only (*b* and *e*), or 100 μg K44.2 (*c* and *f*) and harvested 24 h after the last injection. Hemayoxylin and eosin stained sections are shown in (*a*)–(*c*). Representative melanocytes are indicated by the white arrows. Immunohistochemical staining with the Mel-5 antibody against melanocyte antigen TRP-1 is shown in (*d*)–(*f*). The PBS-injected tissue is shown in (*b*) and (*e*), revealing an intermediate level of melanocytic activity that was also present in the nonimmune murine IgG (not shown). *Scale bar*, 50 μm.
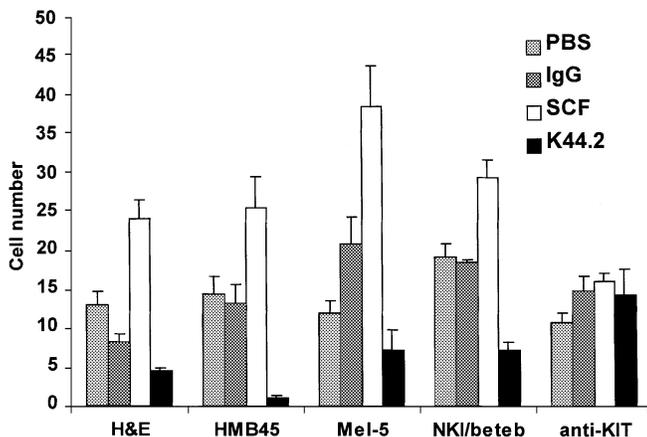
Figure 6. SCF increases and K44.2 decreases the apparent number of cells positive for melanocytic differentiation antigens. Data from experiment 5 are shown. The xenografts were injected once-daily with PBS, nonimmune murine IgG, K44.2, or SCF (2.5 μg) for 4 d. The xenografts were harvested 1 d after the last injection and processed for routine hematoxylin and eosin and immunohistology with HMB45, Mel-5 NKI/beteb, anti-KIT. The data are expressed as cell numbers per 0.5 mm epidermal length (mean ± SEM, n = 4).

the Mel-5 antibody demonstrated marked TRP-1 expression in the SCF-treated tissue (**Figs 5***d*, **6**), whereas inhibition of the KIT receptor with K44.2 (**Figs 5***f*, **6**) resulted in decreased staining and apparent melanocyte loss. The PBS-injected tissue (**Figs 5***b*, *e*, **6**) revealed an intermediate level of melanocytic activity consistent with that expected of normal human skin. The nonimmune murine IgG revealed findings

similar to those in the PBS-injected tissue. KIT did not appear to be as decreased in the SCF-injected tissue in experiment 5 to the same extent as that detected in the earlier experiments, suggesting a degree of recovery at 24 h as compared with that seen 2–4 h after the last SCF injection in the prior experiments. The intensity of the KIT expression appeared to be inversely related to the expression of the melanocytic differentiation antigens. On day 5, although roughly equivalent apparent KIT reactive cell numbers were seen, dramatic shifts in differentiation antigen expression and cell size were apparent. TUNEL labeling of K44.2-treated skin revealed rare apoptotic basal layer cells consistent with melanocytes, but similar cells were also rarely present in some of the other injected tissues. Immunofluorescence evaluation of the K44.2-treated xenografts failed to reveal any cells double-labeled with TRP-1 and Ki67, a finding suggestive of decreased cycling of melanocytes. Due to the paucity of hematoxylin and eosin or TRP-1 identifiable melanocytes in K44.2-injected tissue, however, it was difficult to establish definitively whether there was a greater percentage of apoptotic melanocytes or a decreased percentage of cycling cells in the K44.2-treated tissue. We theorized that, if melano-cytes were undergoing apoptosis, prolonged treatment would result in cell loss that might not be reversible; on the other hand, if melanocytes were merely entering a dormant or less differentiated state, they might recover after antibody treatment was discontinued. In experiment 6, a fragment of the graft was biopsied before treatment and demonstrated the presence of human melanocytes (**Fig 7**). The graft was then treated for 8 d with a daily 25 μl injection of K44.2. Half of the treated graft was harvested on day 9 (1 d after the last dose of antibody) and the other half on day 19 (11 d after the last dose). A marked drop in the number of melanocytes was noted in both the day 9 and the day 19 specimens, with only rare melanocytes noted by either TRP-1, NKI/
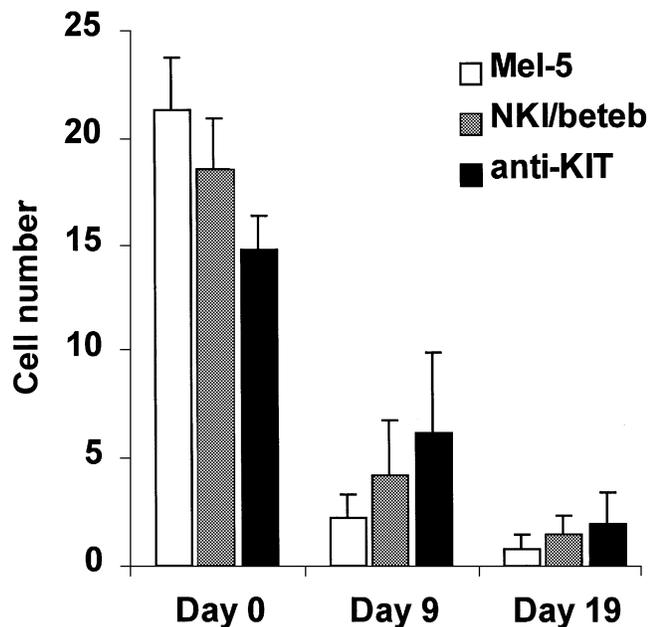
Figure 7. Prolonged K44.2 treatment results in a progressive decrease in the apparent number of melanocytes. Data from experiment 6 are shown. The xenograft was biopsied at day 0 prior to K44.2 injection, at day 9 (1 d after completion of eight once-daily K44.2 injections), and at day 19 (11 d after the last K44.2 injection). The xenograft samples were processed for immunohistology with Mel-5 NKI/beteb, anti-KIT. The data are expressed as cell numbers per 0.5 mm epidermal length (mean ± SEM, n = 4).

beteb, or KIT staining (**Fig 7**). Thus, continued exposure to the KIT inhibitory antibody results in melanocyte loss that does not appear to be reversible, consistent with cell death.

DISCUSSION

We have demonstrated that adult human melanocytes within human skin xenografts respond to manipulation of the SCF/KIT pathway by alteration of both proliferation and differentiation characteristics. These are rather remarkable functions for a single pathway but this dual function is consistent with effects reported for other cell systems including hematopoietic stem cells (Dai *et al*, 1991), mast cells (Tsai *et al*, 1991), and germ cells (Manova *et al*, 1993).

SCF/KIT activation increased expression of the melanocytic differentiation antigens TRP-1 and gp100/pmel17, and conversely KIT inhibition markedly decreased this expression. Because these antigens are implicated in pathways of melanogenesis, this modulation suggests that KIT activation directly affected the critical physiologic function of melanocytes. Melanocyte volume also appeared to increase as a result of SCF stimulation. Interestingly, KIT expression, which we have previously reported to be present on putative quiescent or "precursor" melanocytes (Grichnik *et al*, 1996), appeared to be inversely correlated with the state of SCF/KIT activation, i.e., SCF-treated tissues revealed decreased KIT staining and K44.2-treated specimens showed increased expression. In mast cells, KIT appears to be internalized and degraded after activation by SCF; new expression of the receptor appears to require new synthesis (Shimizu *et al*, 1991). Our results are consistent with negative regulation of KIT expression by activation of this pathway in melanocytes. The melanocytes in the K44.2-treated tissue had markedly decreased TRP-1 expression, suggesting that blockade of the SCF-KIT pathway can inhibit melanization in differentiated melanocytes. Melanoma cell lines in which KIT is downregulated are often amelanotic and have lost TRP-1 activity (Luo *et al*, 1995). Similarly, a melanoma cell line developed from a murine strain homozygous for a mild Kit defect (W^f) is amelanotic (Larue *et al*, 1992). Conversely, SCF treatment of KIT-positive melanoma lines results in a transient increase in DOPA-oxidase activity, suggesting increased melanization capability (Luo *et al*, 1995). The capacity of SCF/KIT activation to drive melanogenesis is also supported by the

human studies, in which skin locally injected with SCF revealed increased melanin and HMB-45 activity (Grichnik *et al*, 1995). Taken together, these data suggest that the SCF/KIT pathway can regulate melanization and that this pathway is functional in xenografted human skin.

SCF has been reported to drive the proliferation of murine melanocyte precursors (Reid *et al*, 1995), and in the presence of dbcAMP or TPA to induce proliferation of human melanocytes *in vitro* (Funasaka *et al*, 1992). In an effort to specifically assess the effect of SCF on proliferation, we evaluated the percentage of melanocytes in the cell cycle as defined by Ki67 expression. The number of cycling melanocytes in SCF-treated xenografts was modest (2–3%), but was several-fold more than in control tissue. For comparison, the epidermal melanocytes of common nevus, atypical nevus, and Spitz's nevus are reported to have 0.31%, 0.67%, and 1.76% Ki67+ cells, respectively, whereas epidermal melanoma cells show a proliferative index of 6.4%, 10.25%, and 13.25% for *in situ*, thin (<0.8 mm), and not as thin (>0.8 mm) tumors, respectively (Ki-S5 antibody, Rudolph *et al*, 1995). Thus treatment with 2.0 μg SCF for 4 d resulted in melanocytic proliferation at a level comparable with that reported for Spitz's nevus but somewhat less than melanoma *in situ*.

Published experiments utilizing ACK2, a specific blocking antibody of SCF activation of Kit (Nishikawa *et al*, 1991), have demonstrated the crucial role of the SCF/Kit pathway for developing murine melanocytes. ACK2 treatment leads to the loss of melanocytes at specific developmental time points, when melanocytes are migrating through the dermis, entering the epidermis, or populating the hair follicles (Yoshida *et al*, 1996). ACK2 inhibition of the Kit receptor appears to result in melanocyte apoptosis (Okura *et al*, 1995). Similarly, our experiments indicate that adult human epidermal melanocytes are quite sensitive to inhibition induced by the K44.2 antibody. The residual human melanocytes within the tissue treated for 4 d with anti-KIT (experiment 5) had small cytoplasmic and nuclear volumes and markedly decreased dendritic processes. After daily treatment for 8 d and an 11 d recovery period, detectable melanocytes remained rare. The mechanism by which this loss occurred seems consistent with apoptosis; however, further studies will be necessary to sort out the specific processes involved in the induction of human melanocyte loss by K44.2.

Our findings suggest that the SCF/KIT pathway may function as a primary mechanism for regulating both proliferation and differentiation of melanocytes. The compartmentalization of this pathway is highly suitable for the homeostatic function of providing protective melanin to the epidermis. SCF on the cell surface of epidermal keratinocytes may permit regulation of adjacent melanocytes within the epidermal compartment, via direct interaction with the melanocyte's KIT receptor. This system may operate in a manner similar to that demonstrated in the testis, where follicle stimulating hormone stimulates the production of SCF by the Leydig cell (Rossi *et al*, 1993), in turn supporting the proliferation of KIT-expressing spermatogonia (Manova *et al*, 1993). This type of control mechanism may allow for tight control of cell types with high proliferative potential, which may include melanocytes within human skin.

Because the SCF/KIT pathway is important for normal melanocytic function, it is possible that alterations of the pathway are responsible for some melanocytic disease processes. Autocrine SCF stimulation of the KIT receptor is an attractive potential mechanism for driving melanocyte proliferation in early melanoma, as has been proposed for several hematologic and solid tumors (Hibi *et al*, 1991; Berdel *et al*, 1992; Sekido *et al*, 1993; Longley *et al*, 1993; Kiss *et al*, 1993; Cohen *et al*, 1994; Nagata *et al*, 1995). Downregulation of KIT has been reported in the later stages of melanoma (Natali *et al*, 1992; Lassam and Bickford, 1992; Mattei *et al*, 1994; Takahashi *et al*, 1995; Ohashi *et al*, 1996; Zakut *et al*, 1993), however, and might permit escape from KIT growth control or, by reducing the expression of differentiation antigens, might allow melanoma cells to evade immunosurveillance. Further research is necessary to decipher the role of the SCF-KIT pathway in melanocytic proliferative processes as well as its role during the early and later stages of melanoma progression. Future pharmacologic therapies based on activation or inhibition of this

pathway may present new treatment options for the treatment of melanocytic disease processes.

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