Murine Cutaneous Mastocytosis and Epidermal Melanocytosis Induced by Keratinocyte Expression of Transgenic Stem Cell Factor

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Summary

The growth and differentiation of mast cells and melanocytes require stem cell factor (SCF), the ligand for the kit receptor tyrosine kinase. SCF may exist as a membrane-bound or soluble molecule. Abnormalities of the SCF-kit signaling pathway, with increased local concentrations of soluble SCF, have been implicated in the pathogenesis of the human disease cutaneous mastocytosis, but have not yet been shown to play a causal role. To investigate both the potential of SCF to cause mastocytosis and its role in epidermal melanocyte homeostasis, we targeted the expression of SCF to epidermal keratinocytes in mice with two different transgenes controlled by the human keratin 14 promoter. The transgenes contained cDNAs that either produced SCF, which can exist in both membrane-bound and soluble forms, or SCF, which remains essentially membrane bound. Murine epidermal keratinocyte expression of membrane-bound/soluble SCF reproduced the phenotype of human cutaneous mastocytosis, with dermal mast cell infiltrates and epidermal hyperpigmentation, and caused the maintenance of a population of melanocytes in the interadnexal epidermis, an area where melanocytes and melanin are found in human skin but where they are not typically found in murine skin. Expression of membrane-bound SCF alone resulted in epidermal melanocytosis and melanin production, but did not by itself cause mastocytosis. We conclude, first, that a phenotype matching that of human mastocytosis can be produced in mice by keratinocyte overproduction of soluble SCF, suggesting a potential cause of this disease. Second, we conclude that keratinocyte expression of membrane-bound SCF results in the postnatal maintenance of epidermal melanocytes in mice. Since the resulting animals have skin that more closely approximates human skin than do normal mice, their study may be more relevant to human melanocyte biology than the study of skin of normal mice.

Key words: mastocytosis • melanocyte development • stem cell factor • c-KIT • mast cell

Melanocytes are present in the interadnexal epidermis in human skin. In contrast, melanocytes in adult murine skin are generally confined to hair follicles, with the exception of rare epidermal melanocytes found in the ears, footpads, and tail (1). A few dermal melanocytes may also be found in mice, mostly in the ears. Melanocyte migration and development, as well as the survival of melanocytes and mast cells, are dependent on expression of the kit protein, a receptor tyrosine kinase encoded by the c-*kit* protooncogene (2–6). The ligand for kit, known as stem cell factor (SCF[1]; also called mast cell growth factor, steel factor, and

[1]*Abbreviations used in this paper:* SCF, stem cell factor; TG, transgene.

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kit ligand) may be produced locally in human skin by epidermal keratinocytes, fibroblasts, and endothelial cells (7, 8). Definitive studies of SCF production in murine skin have not been reported, but transgenic studies using the SCF gene promoter region and β-galactosidase as a reporter gene suggest that, unlike in human skin, postnatal murine cutaneous SCF expression is limited to the dermis and hair follicles, and not found in epidermal keratinocytes (9). The difference in SCF expression between human and murine epidermis could explain the difference in melanocyte distribution in these two species.

SCF may be produced in two isoforms by alternate splicing of exon 6. One isoform lacks exon 6–encoded sequences and exists predominantly as a membrane-bound molecule. The other isoform contains exon 6–encoded sequences, which include a protease-sensitive site (10–19). Cleavage at the protease-sensitive site causes the release of a soluble, bioactive form of SCF. The membrane-bound and soluble forms of SCF have differential effects on melanocyte precursor dispersal and survival (20) and exogenous soluble SCF may produce cutaneous mast cell hyperplasia and cutaneous hyperpigmentation (21–23). In addition, local high concentrations of soluble SCF have been found in lesions of human cutaneous mastocytosis, a disease characterized by dermal accumulations of mast cells and increased epidermal melanin (7, 8, 24). These observations have led to the hypothesis that cutaneous mastocytosis represents a hyperplastic response to locally increased soluble SCF (25). However, clonal proliferations of mast cells containing mutations of c-*KIT*, which result in constitutive activation of kit and a selective growth advantage for the mast cells, have been identified in lesions of some clinical forms of mastocytosis (26), and in the peripheral blood of patients with mastocytosis and hematologic abnormalities (27). Similar mutations have been found in several mast cell lines (28–32). Analysis of these latter data together have suggested that cutaneous mastocytosis may occur as a true primary neoplasm of mast cells (26).

Whether mastocytosis could be caused by overexpression or altered expression of SCF, with mutations occurring as secondary events, or whether c-*KIT* mutations are primary events and neoplastic mast cells induce secondary alterations in the local metabolism of SCF, has not been determined experimentally. Likewise, the reason for a lack of melanocytes in the interadnexal epidermis of murine skin is not known, but may be related to SCF expression.

To reproduce mastocytosis experimentally in the mouse, and to investigate the effects of various forms of SCF on melanocyte migration and development in the epidermis, we developed two types of transgenic mice. One type contained a transgene using the human keratin 14 gene promoter to express epidermal membrane-bound SCF from which the soluble form is spontaneously produced (referred to herein as membrane/soluble SCF). The other type used the same promoter to produce epidermal SCF that normally exists almost exclusively in a membrane-bound form. We found that keratinocyte expression of membrane/soluble SCF resulted in the accumulation of mast cells within the dermis as well as epidermal melanocyte maintenance and pigment production, thereby reproducing the phenotype of mastocytosis without inducing detectable c-*kit* mutations. In contrast, expression of only membrane-bound SCF by epidermal keratinocytes resulted in the maintenance of melanocytes in murine epidermis, thereby mimicking melanocyte growth in human skin, but did not spontaneously produce the mastocytosis phenotype.

Materials and Methods

Transgene Construction. Two murine SCF cDNAs were cloned into constructs containing the human cytokeratin 14 upstream region (33; gift of Dr. E. Fuchs, Howard Hughes Medical Institute, University of Chicago, Chicago, IL; Fig. 1). This promoter causes expression in the skin limited to the basal layers of the interadnexal epidermis and the follicular epithelium. The cDNAs were both full-length clones, containing exon 6–encoded sequences. One cDNA (transgene [TG]1) was unmodified and therefore could produce a membrane-bound protein with the exon 6–encoded protease sensitive site, from which a soluble, bioactive form of SCF could be efficiently generated (10, 11, 34). The product of this transgene will be referred to as membrane/soluble SCF. The second cDNA (TG2) had been previously modified by site-directed mutagenesis, deleting the primary high efficiency cleavage site (between amino acids 164 and 165) and an alternate exon 7–encoded low efficiency cleavage site (found in murine SCF between amino acids 180 and 181). The SCF produced by this transgene therefore exists predominantly as a membrane-bound molecule (membrane SCF; reference 35). Both cDNAs have been previously shown to produce biologically active SCF (35, 36).

Generation and Analysis of Transgenic Animals. 2 μg/ml transgenic DNA in 10 mM Tris (pH 7.5), 0.1 mM EDTA was injected into fertilized oocytes collected from pseudopregnant mice as described (37). At birth, most transgene expressing mice could be identified by distinctive pigmentary phenotypes, as described in Results. Integration of transgenes was verified by PCR of ge-

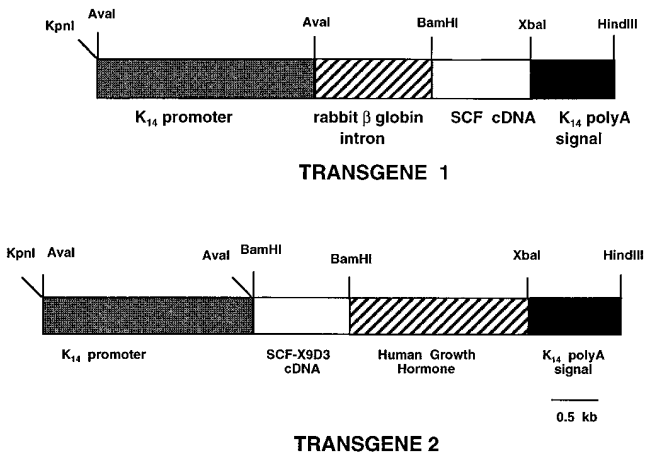


Figure 1. Transgene design. Both transgenes used the human keratin 14 promoter and polyadenylation sequences. TG1 included a rabbit β-globin intron and TG2 included human growth hormone sequences to provide for stability. Neither the β-globin intron nor the human growth hormone sequences produce protein products.

nomic DNA with transgene-specific primers and copy number estimated by Southern blotting of PCR products, followed by autoradiography and densitometry. Skin-specific expression of transgene messenger RNA was confirmed by Northern blotting and by reverse transcription PCR with transgene-specific primers using RNA extracted from representative animals. Transgene expression was quantitated by a ribonuclease protection assay kit (RPA II; Ambion, Austin, TX) according to the manufacturer's directions. In brief, total RNA extracted from mouse skin was hybridized with digoxigenin-labeled single-stranded RNA probes for 23 h at 42°C and digested with RNAseA and RNAseT1, electrophoresed through 5% polyacrylamide/7 M urea. Protected fragments were transferred to Hy^+ membrane (Boehringer Mannheim, Indianapolis, IN), bands were detected by chemiluminescences, and band density was determined by densitometry. Preliminary studies of RNA preparations from each transgenic line were performed to measure β-actin, and the amounts of RNA for SCF mRNA determinations were adjusted for comparison. RNA was also used with reverse transcription and PCR for direct amplimer sequencing of c-*kit* mRNA sequences in regions that could contain known activating mutations, as previously described (26). The primers used were 5′ CAAATC/GCATCCC/TCACACCCTGTTCAC and 5′ CCATAAGCAGTTGCCTCAAC, which bind to nucleotides 1568→1593 and 1854→1835, and 5′ TGTATTCACAGAGACTTGGC and 5′ AAAATCCCATAGGACCAGAC binding to nucleotides 2384→2403 and 2595→2576. These regions contain the codons with both of the activating mutations, codon 559 and codon 814, respectively, which have been described in human mastocytosis and in a murine mast cell line (5, 28).

TG1, containing the full-length unmodified SCF cDNA (membrane/soluble SCF), was injected into 100 F1 oocytes (C57 BL6 × SLJ), which were implanted into six host mothers, resulting in four independent hyperpigmented mice, all of which were positive for the transgene, and 40 other littermates that were pigmentary phenotype– and transgene–negative by PCR.

Oocytes for TG2 (membrane SCF) were F1 (C57BL/6J female × SLJ/J male) and the offspring could be black, agouti, or white. Injection of 40 embryos and implantation into 6 host mothers generated 48 pups, 21 of which were positive for integration by PCR. Of the 25 founder mice identified by PCR with the transgene-specific primers, 3 were black, 13 were agouti, and 9 were white. Five PCR-positive mice (three agouti and two black) showed a clearly identifiable pigmentary phenotype. Given the inability of white mice to produce normal cutaneous pigment, it is possible that there were also white founders that expressed the transgene without the production of an obvious change in pigment. Backcrossing of phenotype-positive black and agouti founders to C57 BL/6 mice produced uniform pigmentary changes, described in the Results section.

Histology, Immunohistochemistry, and Electron Microscopy. Tissues from transgenic and littermate mice were fixed in formalin and embedded in paraffin or polyester wax, sectioned, and stained with hematoxylin and eosin, azure blue, alcian blue, or Giemsa's stain according to standard techniques (37–39). Immunofluorescence studies were performed on polyester wax-embedded sections or frozen sections, also using standard techniques. Antibodies included anti-S100 (rabbit anti–cow S100, prediluted; Dako Corp., Carpinteria, CA), and the ACK2 and ACK4 monoclonals (rat anti–mouse c-*kit* [40] at 20 μg/ml). Controls included omission of the primary antibody or the use of isotype-matched monoclonal antibodies of irrelevant specificity. Electron microscopy was done as previously described (41).

Results

Dermal Mast Cells Accumulate in the Presence of Membrane/soluble Keratinocyte SCF. Sections of skin from all mice producing membrane/soluble SCF (TG1) showed increased mast cells in the dermis (Fig. 2). In newborn TG1-positive mice, the mast cells were superficial near the dermal–epidermal junction, close to the epidermal source of soluble SCF (Fig. 2 *a*). In older mice, the mast cells filled the papillary dermis in some areas, but were also present in the reticular dermis, in a pattern identical to that of human mastocytosis (Fig. 2, *b–d*). Electron microscopic analysis confirmed the presence of numerous mast cells with characteristic granules within the dermis of the TG1-positive animals and also showed that some of the heavily pigmented cells within the dermis of TG1-positive mice were melanocytes (Fig. 3). Mast cells were relatively rare and dermal melanocytes were not detected in the body wall skin of nontransgenic littermates and in TG2-positive animals of equivalent age. These observations were true across a wide range of copy numbers and levels of SCF mRNA expression (Fig. 4). Since the keratin 14 promoter is properly expressed in the skin only by keratinocytes, and since the production of only membrane-bound keratinocyte SCF did not spontaneously result in increased dermal mast cells in TG2-positive animals, keratinocyte production of the soluble form of SCF appears to be able to cause cutaneous mastocytosis in mice.

Known Activating c-kit Mutations Are Not Identified in Transgenic Mice. Reverse transcriptase PCR analysis of mRNA extracted from the skin of both types of transgenic mice showed normal murine c-*kit* sequences with no evidence of activating mutations in two of the TG1 mice and one of the TG2 mice. Since the majority of the c-*kit* mRNA molecules in the TG2 mouse samples were derived from mast cells and melanocytes, this technique would identify mutations if they were expressed by a significant percentage of these cells (26).

SCF Transgenic Mice Are Hyperpigmented. Targeted expression of each of the SCF transgenes in murine skin caused a similar, distinctive pigmented phenotype. The pigment responsible for the coat color of normal mice resides in the hair follicles and hair shafts, not in the epidermis. The transgenic mice, however, developed prominent epidermal pigmentation (Fig. 5). Transgene-positive animals could be identified by increased pigment at birth. By ~21 d of age, the phenotypes were well established; phenotype-positive animals showed pigmentation of most of the skin as well as increased coat pigment. Extensive pigmentation was noted in a number of areas including the nose, mouth, ears, paws, and external genitalia when compared with normal littermate controls. There was enough individual variation in pigmentation so that no clear correlation between the level of pigmentation and the levels of transgenic expression could be shown. All transgenic animals showed similar degrees of pigmentation regardless of transgene type, copy number, or levels of SCF mRNA expression. In addition to the epidermal pigmentation, the three TG2-positive agouti founders

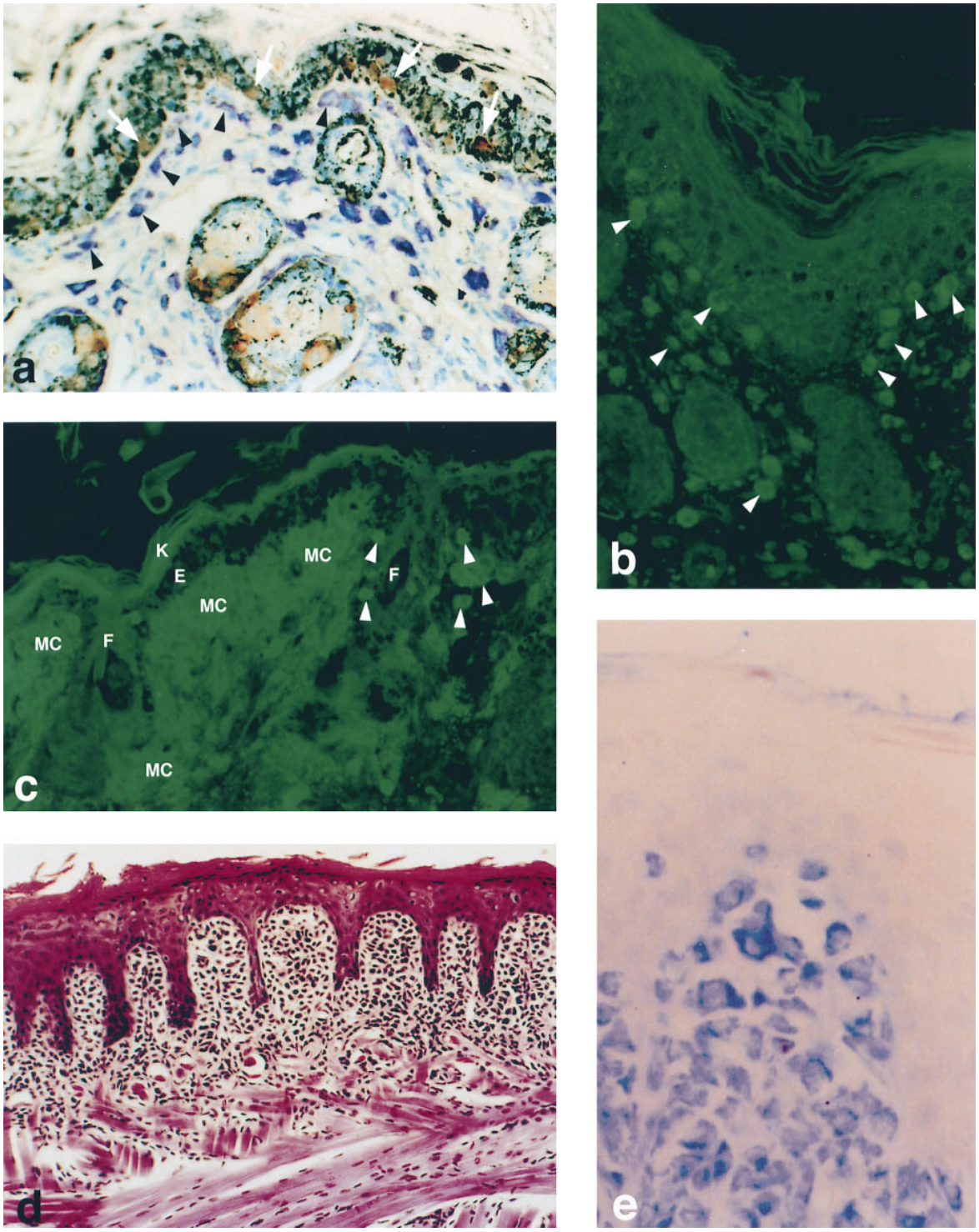


Figure 2. Increased mast cells in mice expressing epidermal membrane and soluble SCF (TG1). (*a*) Numerous mast cells are demonstrated in the superficial dermis of body wall skin of newborn mice bearing TG1 (membrane/soluble SCF) using an immunoperoxidase/alcian blue technique that stains mast cell granules metachromatically purple. Note the apposition of mast cells (*arrowheads*) to basalar keratinocytes, the source of SCF. Immunoperoxidase with an anti-S100 antibody in this preparation also demonstrates melanocytes as brown-staining cells in the basalar layers of epidermis and follicular epithelium (*white arrows*). Sebocytes are seen as large, round, lightly S-100(+) cells in the follicular epithelium. Melanin pigment is stained black in this preparation. (*b*) Immunofluorescence with anti-kit antibodies highlights kit-expressing dermal mast cells (*arrowheads*) in body wall skin of newborn (TG1 membrane/soluble SCF) mouse. (*c*) Anti-kit antibody immunofluorescence shows mast cells crowded in the papillary dermis and extending into the upper reticular dermis and body wall skin of a 21-d-old, TG1-positive mouse. *MC*, confluent mast cells; *arrowheads*, individual and small clusters of mast cells; *E*, epidermis; *F*, follicles; *K*, keratin layer. (*d*) Hematoxylin and eosin–stained sections show mast cells filling the superficial corium in section of tongue from a 21-d-old, TG1-positive mouse. The lack of abundant melanocytes and melanophages in this anatomic site allows easy visualization of the mast cells. This histologic picture is identical to that seen in human cutaneous mastocytosis. (*e*) Alcian blue–stained serial section of tongue shows metachromatic granules in mast cells of a 21-d-old, TG1-positive mouse.

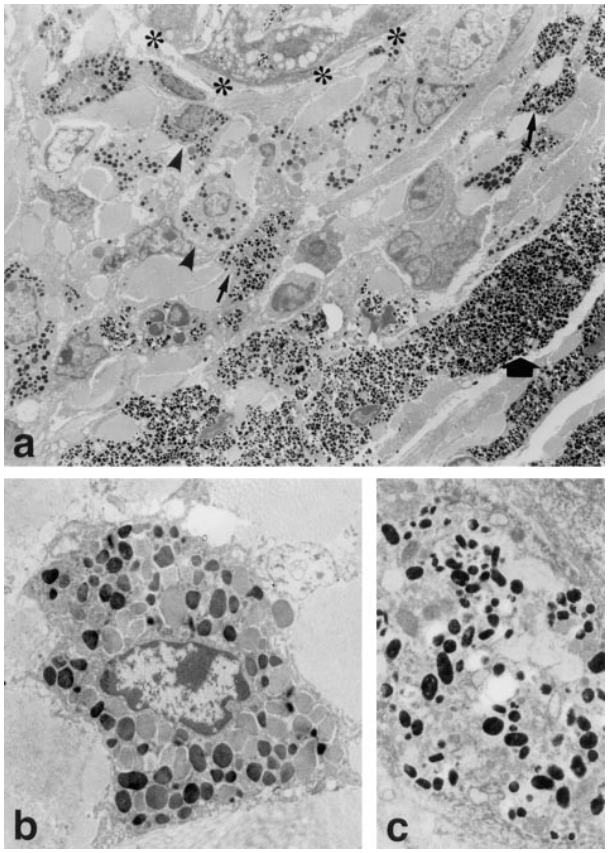


Figure 3. Electron microscopy confirms the presence of melanocytes and mast cells in transgenic mice. (*a*) TG1 mouse with membrane/soluble epidermal SCF has numerous dermal mast cells (*arrowheads*) as well as dermal melanocytes (*arrows*). *Asterisks*, the boundary of the dermis and hair follicle. Higher magnification images of mast cell and melanocyte are shown in *b* and *c*, respectively. Original magnifications: (*a*) 2,750, (*b*) 9,000, (*c*) 11,750.

showed thin black transverse strips, consistent with the pigment distribution of the allophenic mice described by B. Mintz (pictures not shown; reference 42).

Numerous Melanocytes Are Maintained in the Skin of Transgenic Mice. The increased pigmentation of the skin of the transgene-positive mice of both types is attributable to the presence of intraepidermal melanocytes, and to the epidermal melanin produced by those cells. Intraepidermal melanocytes can be identified in hematoxylin and eosin–stained sections as cells in the basalar layers surrounded by clear halos (Fig. 6, *a* and *b*) or in immunoperoxidase preparations by their expression of S-100 protein. Immunohistochemical analysis of animals expressing each of the transgenes showed numerous S-100(+) intraepidermal melanocytes (also see Fig. 2 *a*). These melanocytes can be differentiated from Langerhans cells, which also express S-100 protein, because melanocytes are in the basal layers and Langerhans are in the suprabasal layers. Melanocytes can also be differentiated from Langerhans cells by their expression of the kit protein, the receptor for SCF, which is not expressed by Langerhans cells. Staining of transgenic animal skin with anti-kit antibody identified well-developed dendritic cells

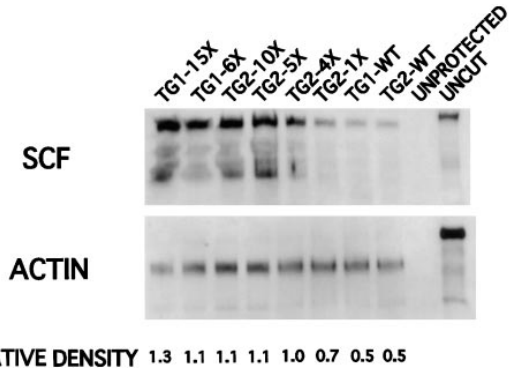


Figure 4. Transgenic phenotypes are stable across a wide range of gene expression levels. This figure compares the transgene copy number determined by PCR, with SCF mRNA expression as determined by RNAse protection assay, in lines from different founders. The relative density of SCF bands was determined by dividing the mean density of the SCF band by the density of an actin band derived from an identical aliquot of RNA. Probe templates were 384 bases in length for SCF (40 base pairs of promoter sequence and 342 bases complimentary to nucleotides 814–1156 of murine SCF mRNA; reference 5). A β-actin probe was used as a control and to allow standardization between RNA preparations from different mice. The β-actin probe length was 310 bases, 227 bases of which are complementary to murine β-actin mRNA. The probe was purchased from Ambion (pTR1–β-actin–mouse anti-sense control template). Note the overlap between TG2 (4×, 5×, 10×) and TG1 (6×).

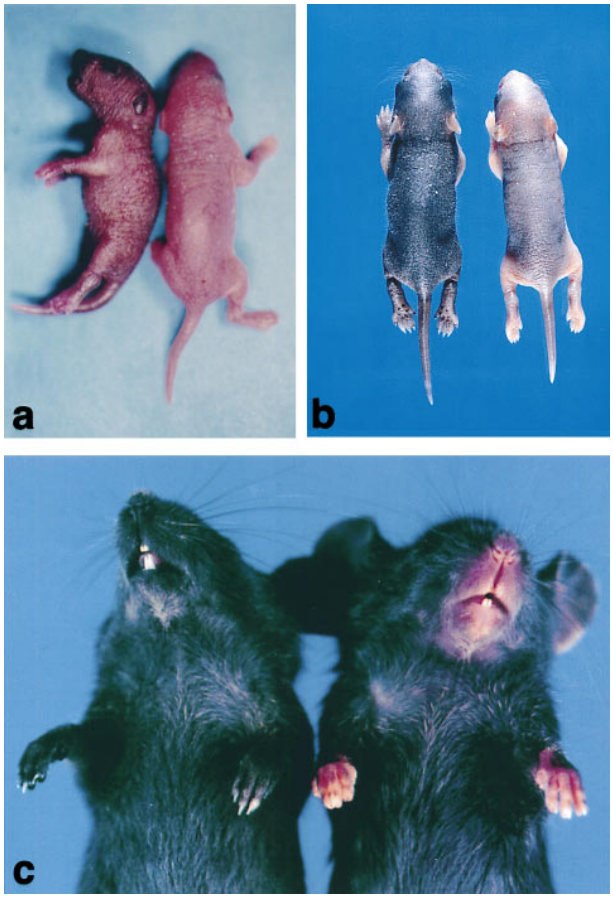
within the basalar layers of the epidermis and follicular epithelium, consistent with melanocytes (Figs. 6 *c* and 2 *b*).

Histologic examination confirmed the presence of pigment within the epidermis of both TG1 and TG2 phenotype–positive mice from all sites examined, including the ears, tail, footpads, and body wall (Fig. 6 *a*). In addition, TG1-positive mice showed many pigmented cells within the dermis. Pigmentary abnormalities were not observed in transgene-negative littermates. Only slight epidermal pigment was identified in these control mice, and mostly in non–hair-bearing areas like the footpad and tail. Although pigment patterns were stable throughout much of the adult life of the mice, an occasional TG1 (membrane/soluble SCF) mouse developed patchy areas of depigmentation, mostly in the ears, associated with loss of epidermal melanocytes and increased pigment incontinence. This phenomenon was not observed in the membrane SCF mice.

Electron microscopy confirmed the presence of numerous melanocytes within the epidermis of both types of transgenic mice (Fig. 7). Pigmented keratinocytes, similar to those seen in the epidermis of humans, were also present in the interadnexal epidermis of the transgenic mice. Intraepidermal melanocytes and pigmented keratinocytes were extremely rare in control mice.

Discussion

The human disease mastocytosis is a heterogeneous group of conditions characterized by increased numbers of mast cells in various organs, most commonly the dermis (24). Mastocytosis involving the skin is also characterized



by increased epidermal melanin, produced by melanocytes. Since melanocytes are neuroectodermally derived cells that migrate to the epidermis through patterns distinct from those of mesodermally derived mast cells, the colocalization of pigmentary abnormalities and mast cells in lesions of cutaneous mastocytosis implies the involvement of local factors. It has been suggested that the proliferation of mast cells in human mastocytosis is a reactive phenomenon rather than a true neoplastic process (7, 25) based upon the following three observations: (*a*) both melanocytes and mast cells express KIT and respond to SCF, (*c*) the injection of soluble SCF can cause epidermal pigmentation and dermal mast cell accumulation (21, 23, 43), and (*c*) increased amounts of soluble SCF have been identified in lesions of cutaneous mastocytosis (7, 8). In apparent conflict with this hypothesis is the fact that c-*KIT* mutations resulting in constitutive activation of KIT have been identified in multiple lesions of human mastocytosis, evidence that the mast cells in some cases of human mastocytosis represent a true proliferating neoplastic clone (26). However, recent studies

Figure 5. Epidermal SCF causes hyperpigmentation of murine skin. (*a*) Newborn mouse expressing membrane/soluble SCF (TG1, *left*) shows obvious hyperpigmentation compared with nontransgenic littermate (*right*). (*b*) TG2-positive mouse overexpressing membrane-bound epidermal SCF shows a similar phenotype with generalized hyperpigmentation, which is most discernible in the hairless areas, and which is maintained in adult life. 3-wk-old TG2-positive mouse (*left*) and nontransgenic littermate (*right*).

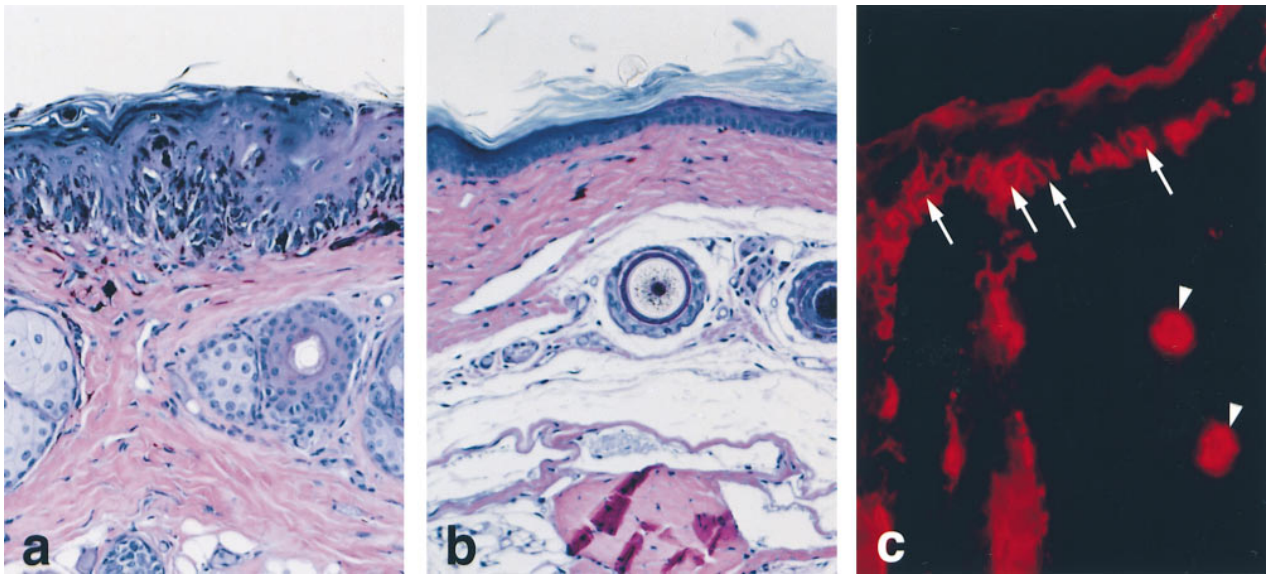


Figure 6. Intraepidermal melanocytes are increased in transgenic mice. (*a*) Tail skin section from 21-d-old mouse expressing epidermal membrane-bound SCF (TG2) shows mild epidermal hyperplasia and a markedly increased number of melanocytes, identified as cells surrounded by clear halos, mostly at the dermal–epidermal junction. These mice also show extensive black epidermal melanin pigment (original magnification: 400). (*b*) Note the lack of both basalar melanocytes and epidermal pigment in the skin of the transgene (−) littermate control mouse (C57 black 6; original magnification: 400). (*c*) Epidermal melanocytes express kit protein. Immunofluorescence staining with anti-kit antibody and Texas red–labeled secondary antibody demonstrates confluent dendritic cells in the epidermal basalar layer of mice expressing membrane-bound SCF (TG2, *arrows*). These cells correspond to the S-100 protein (+) basalar dendritic cells seen in Fig. 2 *a*. Note two strongly kit-positive solitary mast cells in the dermis (*arrowheads*; original magnification: 400). Light staining of dendritic melanocytes can also be seen in the epidermis of TG1-positive mice (see Fig. 2 *b*).

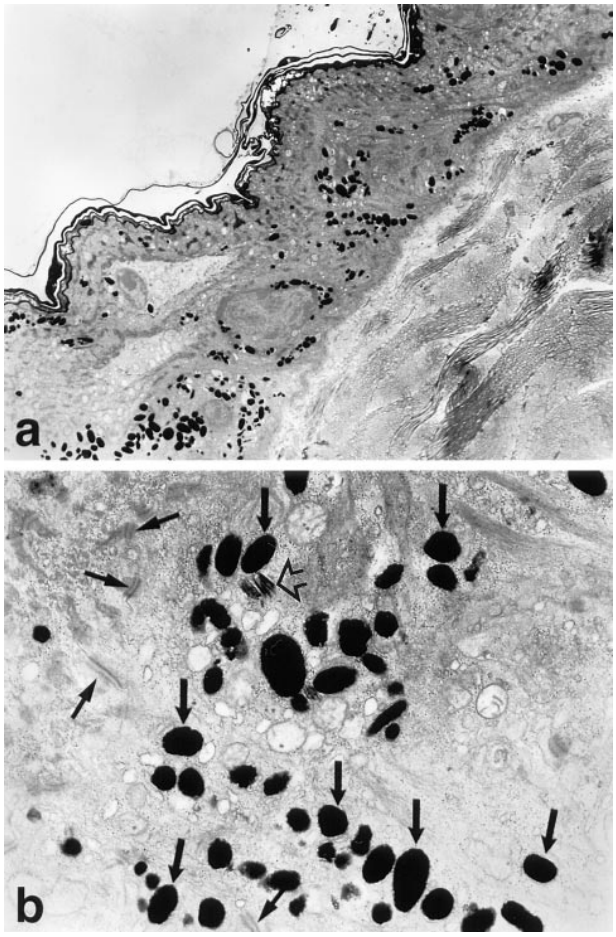


Figure 7. Electron microscopy confirms the presence of epidermal melanocytes in both types of transgenic mice. (*a*) Electron microscopy shows numerous keratinocytes containing phagocytized melanin granules in the interadnexal epidermis of mice expressing membrane-bound epidermal SCF (original magnification: 3500). (*b*) Epidermal melanosomes, some marked with large arrows, are present in both keratinocytes and melanocytes. Premelanosomes, marked with the open arrows, demonstrate the presence of a melanocyte. Note keratinocyte hemidesmosomes (*small arrows*), which confirm the location of the melanocyte within the epidermis (16; original magnification: 320).

suggest that only some clinical forms of human mastocytosis are associated with these mutations (Longley, B.J., unpublished observations), so the possible causes of mastocytosis in the clinical forms not associated with c-*KIT* mutations remain to be elucidated. In this study, we were able to reproduce the phenotype of mastocytosis in mice by expression of soluble SCF by murine epidermal keratinocytes. None of the known activating c-*kit* mutations were identified in the proliferating mast cells, showing that activating mutations are not necessary to produce the phenotype of this disease. These observations highlight a potential cause of mastocytosis in the absence of activating c-*KIT* mutations. Although the work reported in this manuscript fulfills one of Koch's criteria for establishing the cause of a disease, e.g., the reproduction of the disease in animals, it should be noted that murine mast cells differ considerably from human mast cells with regard to their growth requirements and that there are other potential growth factors and cytokines besides SCF that could contribute to the development of mastocytosis.

Melanocytes are maintained in human epidermis throughout life. In normal mice DOPA reaction–positive cells (melanoblasts and melanocytes) are found in the epidermis at birth, but their number decreases from postnatal day 4 and is severely reduced after 1 mo of age (44). One possible explanation for the maintenance of epidermal melanocytes in human skin, and the difference between the distribution of melanocytes in adult human and murine skin, could be expression of epidermal SCF. Human epidermal keratinocytes produce SCF (7, 8, 45), but the SCF gene does not appear to be expressed in murine epidermis (9). The results presented here show that SCF expression by murine epidermal keratinocytes causes the maintenance and stimulation of epidermal melanocytes throughout life. These data support the hypothesis that the decrease in melanocyte numbers in the postnatal mouse epidermis is due to a lack of local SCF expression. In combination with the fact that the soluble SCF produced by Sl/Sl^d mice is insufficient to support normal melanocyte survival and the observations that membrane-bound SCF promotes longer lasting kit activation and increased survival of kit-dependent cells in the hematopoietic system (1, 2, 10–12, 35), our data suggest that it is specifically the membrane-bound form of SCF that is crucial for melanocyte survival and function.

It is interesting to note that none of the animals expressing either of the transgenes described in this paper have developed melanoma to date, a finding that supports previous observations that stimulation of the kit tyrosine kinase receptor does not appear to promote the development of melanocytic tumors (46). It also seems likely that the animals described in this paper, or animals derived from them, will be useful in the study of cutaneous mastocytosis and epidermal melanocyte biology.

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References

1. Silvers, W.K. 1979. The coat colors of mice: a model for mammalian gene action and interaction. Springer-Verlag, Inc., New York. 4–5.
2. Mayer, T.C. 1970. A comparison of pigment cell development in albino, steel, and dominant-spotting mutant mouse embryos. *Dev. Biol.* 23:297–309.
3. Russell, E.S. 1979. Hereditary anemias of the mouse: a review for geneticists. *Adv. Genet.* 20:357–459.
4. Yarden, Y., W.J. Kuang, T. Yang-Feng, L. Coussens, S. Munemitsu, T.J. Dull, E. Chen, J. Schlessinger, U. Francke, and A. Ullrich. 1987. Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. *EMBO (Eur. Mol. Biol. Organ.) J.* 6:3341–3351.
5. Qiu, F.H., P. Ray, K. Brown, P.E. Barker, S. Jhanwar, F.H. Ruddle, and P. Besmer. 1988. Primary structure of c-kit: relationship with the CSF-1/PDGF receptor kinase family—oncogenic activation of v-kit involves deletion of extracellular domain and C terminus. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:1003–1011.
6. Geissler, E.N., M.A. Ryan, and D.E. Housman. 1988. The dominant-white spotting (W) locus of the mouse encodes the c-kit proto-oncogene. *Cell.* 55:185–192.
7. Longley, B.J., Jr., G.S. Morganroth, L. Tyrrell, T.G. Ding, D.M. Anderson, D.E. Williams, and R. Halaban. 1993. Altered metabolism of mast-cell growth factor (c-kit ligand) in cutaneous mastocytosis. *N. Engl. J. Med.* 328:1302–1307.
8. Weiss, R.R., D. Whitaker-Menezes, J. Longley, J. Bender, and G.F. Murphy. 1995. Human dermal endothelial cells express membrane-associated mast cell growth factor. *J. Invest. Dermatol.* 104:101–106.
9. Yoshida, H., S.-I. Hayashi, L.D. Shultz, K.I. Yamamura, S. Nishikawa, S.-I. Nishikawa, and T. Kunisada. 1996. Neural and skin cell specific expression pattern conferred by Steel factor regulatory sequence in transgenic mice. *Dev. Dyn.* 207: 222–232.
10. Anderson, D.M., S.D. Lyman, A. Baird, J.M. Wignall, J. Eisenman, C. Rauch, C.J. March, H.S. Boswell, S.D. Gimpel, D. Cosman, and D.E. Williams. 1990. Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. *Cell.* 63: 235–243.
11. Zsebo, K.M., J. Wypych, I.K. McNiece, H.S. Lu, K.A. Smith, S.B. Karkare, R.K. Sachdev, V.N. Yuschenkoff, N.C. Birkett, L.R. Williams, et al. 1990. Identification, purification, and biological characterization of hematopoietic stem cell factor from buffalo rat liver–conditioned medium. *Cell.* 63:195–201.
12. Flanagan, J.G., and P. Leder. 1990. The kit ligand: a cell surface molecule altered in steel mutant fibroblasts. *Cell.* 63: 185–194.
13. Onoue, H., Y. Ebi, H. Nakayama, X.M. Ru, Y. Kitamura, and J. Fujita. 1989. Suppressive effects of Sl/Sld mouse embryo-derived fibroblast cell lines on diffusible factor–dependent proliferation of mast cells. *Blood.* 74:1557–1562.
14. Anderson, D.M., D.E. Williams, R. Tushinski, S. Simpel, J. Eisenman, L.A. Cannizzaro, M. Aronson, C.M. Croce, K. Huebner, D. Cosman, and S.D. Lyman. 1991. Alternate splicing of mRNAs encoding human mast cell growth factor and localization of the gene to chromosome 12q22-q24. *Cell Growth Differ.* 2:373–378.
15. Lu, H.S., C.L. Clogston, J. Wypych, P.R. Fausset, S. Lauren, E.A. Mendiaz, K.M. Zsebo, and K.E. Langley. 1991. Amino acid sequence and post-translational modification of stem cell factor isolated from buffalo rat liver cell–conditioned medium. *J. Biol. Chem.* 266:8102–8107.
16. Flanagan, J.G., D.C. Chan, and P. Leder. 1991. Transmembrane form of the kit ligand growth factor is determined by alternative splicing and is missing Sld mutant. *Cell.* 64:1025–1035.
17. Brannan, C.I., S.D. Lyman, D.E. Williams, J. Eisenman, D.M. Anderson, D. Cosman, M.A. Bedell, N.A. Jenkins, and N.G. Copeland. 1991. Steel-Dickie mutation encodes a c-Kit ligand lacking transmembrane and cytoplasmic domains. *Proc. Natl. Acad. Sci. USA.* 88:4671–4674.
18. Zsebo, K.M., D.A. Williams, E.N. Geissler, V.C. Broudy, F.H. Martin, H.L. Atkins, R.-Y. Hsu, N.C. Birkett, K.H. Okino, D.C. Murdock, et al. 1990. Stem cell factor is encoded at the Sl locus of the mouse and is the ligand for the c-kit tyrosine kinase receptor. *Cell.* 63:213–224.
19. Huang, E.J., K.H. Nocka, J. Buck, and P. Besmer. 1992. Differential expression and processing of two cell associated forms of the kit-ligand: KL-1 and KL-2. *Mol. Biol. Cell.* 3: 349–362.
20. Wehrle-Haller, B., and J.A. Weston. 1995. Soluble and cell-bound forms of steel factor activity play distinct roles in melanocyte precursor dispersal and survival on the lateral neural crest migration pathway. *Development.* 121:731–742.
21. Tsai, M., L.-S. Shih, G.F.J. Newlands, T. Takeishi, K.E. Langley, K.M. Zsebo, H.R.P. Miller, E.N. Geissler, and S.J. Galli. 1991. The rat c-kit ligand, stem cell factor, induces the development of connective tissue–type and mucosal mast cells in vivo. Analysis by anatomical distribution, histochemistry, and protease phenotype. *J. Exp. Med.* 174:125–131.
22. Harrist, T.J., J.J. Costa, G.D. Demetri, A.M. Dvorak, D.F. Hayes, E.A. Merica, D. Menchaca, A.J. Gringeri, and S.J. Galli. 1995. Recombinant human stem cell factor (SCF) (c-kit ligand) promotes melanocyte hyperplasia and activation in vivo. *Lab. Invest.* 72:48A. (Abstr.)
23. Costa, J.J., G.D. Demetri, T.J. Harrist, A.M. Dvorak, D.F. Hayes, E.A. Merica, D.M. Menchaca, A.J. Gringeri, L.B. Schwartz, and S.J. Galli,. 1996. Recombinant human stem cell factor (KIT ligand) promotes human mast cell and melanocyte hyperplasia and functional activation in vivo. *J. Exp. Med.* 183:2681–2686.
24. Longley, B.J., T.P. Duffy, and S. Kohn. 1995. The mast cell and mast cell disease. *J. Am. Acad. Dermatol.* 32:545–561.
25. Longley, J. 1994. Is mastocytosis a mast cell neoplasia or a reactive hyperplasia? *Ann. Med.* 26:115–116.
26. Longley, B.J., L. Tyrrell, S.-Z. Lu, Y.-S. Ma, K. Langley, T.G. Ding, T. Duffy, P. Jacobs, L.H. Tang, and I. Modlin. 1996. Somatic c-KIT activating mutation in urticaria pigmentosa and aggressive mastocytosis; establishment of clonality in a human mast cell neoplasm. *Nat. Genet.* 12:312–314.
27. Nagata, H., A.S. Worobec, C.K. Oh, B.A. Chowdhury, S.

Tannenbaum, Y. Suzuki, and D.D. Metcalfe. 1995. Identification of a point mutation in the catalytic domain of the protooncogene c-kit in peripheral blood mononuclear cells of patients who have mastocytosis with an associated hematologic disorder. *Proc. Natl. Acad. Sci. USA*. 92:10560–10564.

28. Furitsu, T., T. Tsujimura, T. Tono, H. Ikeda, H. Kitayama, U. Koshimizu, H. Sugahara, J.H. Butterfield, L.K. Ashman, Y. Kanayama, et al. 1993. Identification of mutations in the coding sequence of the proto-oncogene c-KIT in a human mast cell leukemia cell line causing ligand-independent activation of c-KIT product. *J. Clin. Invest.* 92:1736–1744.
29. Kitayama, H., Y. Kanakura, T. Furitsu, T. Tsujimura, K. Oritani, H. Ikeda, H. Sugahara, H. Mitsui, Y. Kanayama, and Y. Kitamura. 1995. Constitutively activating mutations of c-kit receptor tyrosine kinase confer factor–independent growth and tumorigenicity of factor-dependent hematopoietic cell lines. *Blood*. 85:790–798.
30. Hashimoto, K., T. Tsujimura, M. Kimura, K. Tohya, M. Morimoto, H. Kitayama, Y. Kanakura, and Y. Kitamura. 1996. Transforming and differentiation-inducing potential of constitutively activated c-kit mutant genes in the IC-2 murine interleukin-3–dependent mast cell line. *Am. J. Pathol.* 148:189–200.
31. Tsujimura, T., M. Morimoto, K. Hashimoto, Y. Moriyama, H. Kitayama, Y. Matsuzawa, Y. Kitamura, and Y. Kanakura. 1996. Constitutive activation of c-kit in FMA3 murine mastocytoma cells caused by deletion of seven amino acids at the juxtamembrane domain. *Blood*. 87:273–283.
32. Tsujimura, T., T. Furitsu, M. Morimoto, Y. Kanayama, S. Nomura, Y. Matsuzawa, Y. Kitamura, and Y. Kanakura. 1995. Substitution of an aspartic acid results in constitutive activation of c-kit receptor tyrosine kinase in a rat tumor mast cell line RBL-2H3. *Int. Arch. Allergy Immunol*. 106:377–385.
33. Vassar, R., M. Rosenberg, S. Ross, A. Tyner, and E. Fuchs. 1989. Tissue-specific and differentiation-specific expression of a human K14 keratin gene in transgenic mice. *Proc. Natl. Acad. Sci. USA*. 86:1563–1567.
34. Williams, D.E., J. Eisenman, A. Baird, C. Rauch, K. Van Ness, C.J. March, L.S. Park, U. Martin, D.V. Mochizuki, and H.S. Boswell. 1990. Identification of a ligand for the c-kit proto-oncogene. *Cell*. 63:167–174.
35. Majumdar, M.K., L. Feng, E. Medlock, D. Toksoz, and D.A. Williams. 1994. Identification and mutation of primary and secondary proteolytic cleavage sites in murine stem cell factor cDNA yields biologically active, cell-associated protein. *J. Biol. Chem*. 269:1237–1242.
36. Yasunaga, M., F.H. Wang, T. Kunisada, S. Nishikawa, and S.I. Nishikawa. 1995. Cell cycle control of c-kit -1 IL-7R1 B precursor cells by two distinct signals derived from IL-7 receptor and c-kit in a fully defined medium. *J. Exp. Med*. 182: 315–323.
37. Kunisada, T., H. Yoshida, M. Ogawa, L.D. Shultz, and S.-I. Nishikawa. 1996. Characterization and isolation of melanocyte progenitors from mouse embryos. *Dev. Growth Differ*. 38:87–97.
38. Yoshida, H., T. Kunisada, and S.-I. Nishikawa. 1996. Distinct stages of melanocyte differentiation revealed by analysis of nonuniform pigmentation patterns. *Development*. 122: 1207–1214.
39. Scott, J.E., and R.T. Mowry. 1970. Alcian blue—a consumer's guide. *J. Histochem. Cytochem*. 18:842.
40. Nishikawa, S., M. Kusakabe, K. Yoshinaga, M. Ogawa, S.-I. Hayashi, T. Kunisada, T. Era, T. Sakakura, and S.-I. Nishikawa. 1991. In utero manipulation of coat color formation by a monoclonal anti–c-kit antibody: two distinct waves of c-kit–dependency during melanocyte development. *EMBO (Eur. Mol. Biol. Organ.) J.* 10:2111–2118.
41. Okura, M., H. Maeda, S.-I. Nishikawa, and M. Mochizuki. 1995. Effects of monoclonal anti–c-kit antibody (ACK2) on melanocytes in newborn mice. *J. Invest. Dermatol.* 105:322–328.
42. Bradl, M., L. Larue, and B. Mintz. 1991. Clonal coat color variation due to a transforming gene expressed in melanocytes of transgenic mice. *Proc. Natl. Acad. Sci. USA*. 88:6447–6451.
43. Grichnik, J.M., J. Crawford, F. Jimenez, J. Kurtzberg, M. Buchanan, S. Blackwell, R.E. Clark, and M.G. Hitchcock. 1995. Human recombinant stem–cell factor induces melanocytic hyperplasia in susceptible patients. *J. Am. Acad. Dermatol.* 33:577–583.
44. Hirobe, T. 1984. Histochemical survey of the distribution of the epidermal melanoblasts and melanocytes in the mouse during fetal and postnatal periods. *Anat. Rec*. 208:589–594.
45. Hamann, K., N. Haas, J. Grabbe, and B.M. Czarnetzki. 1995. Expression of stem cell factor in cutaneous mastocytosis. *Br. J. Dermatol*. 133:203–208.
46. Funasaka, Y., T. Boulton, M. Cobb, Y. Yarden, B. Fan, S.D. Lyman, D.E. Williams, D.M.Anerson, R. Zakut, Y. Mishima, and R. Halaban. 1992. C-kit–kinase induces a cascade of protein tyrosine phosphorylation in normal human melanocytes in response to mast cell growth factor and stimulates mitogen-activated protein kinase but is down-regulated in melanomas. *Mol. Biol. Cell.* 3:197–209.