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Immunohistochemical localisation of stem cell factor (SCF) with comparison of its receptor c-Kit proto-oncogene product (c-KIT) in melanocytic tumours

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Abstract In order to characterise the distribution and role of stem cell factor (SCF), a recently-reported growth factor for normal melanocytes, we carried out an immunohistochemical study on benign and malignant melanocytic tumours with a comparison with the presence of its receptor c-Kit proto-oncogene product (c-KIT). In normal skin, SCF was mainly observed in endothelial cells of blood vessels but not frequently in basal melanocytes, whereas c-KIT was predominantly localised in tissue mast cells. In benign neoplastic melanocytes (common melanocytic naevi), localisation of SCF and c-KIT was complementary: SCF was mostly found in dermal naevus cells while c-KIT was revealed in epidermal naevus cells, although the expression of the latter antigen was not frequent. Malignant melanoma cells showed less frequent expression of these antigens than those in benign lesions. Of five cultured melanoma cell lines, SCF was observed in only one, and c-KIT was not found in any melanoma cells. No quantitative or qualitative alterations assessed by Western blot analysis were induced in the presence of phenotypic modifiers (sodium butyrate and HMBA). Present data suggest that loss of SCF expression in neoplastic melanocytes is commonly associated with malignant transformation of pigment cells rather than loss of its receptor c-KIT.

Key words c-KIT · Immunohistochemistry · Malignant transformation · Melanocyte · SCF

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Introduction

Malignant transformation of pigment cells occurs in a multistep progression including loss of dependence on growth and/or phenotypic regulators for melanocytes. In addition to basic fibroblast growth factor (bFGF) [2, 6] and endothelin [26], hepatocyte growth factor (HGF) [11] and stem cell factor (SCF) [5] or mast cell growth factor (MGF) are new candidate growth stimulators for normal melanocytes. Interaction with receptors for these ligands, the proto-oncogenes c-Met and c-Kit, respectively, results in regulation of melanocyte proliferation and/or differentiation, mediated through signal transduction and protein phosphorylation [4]. Previous reports [7, 16] showed that the effects of HGF and SCF occasionally differ between benign and malignant melanocytes. For example, Halaban et al. [7] have shown that HGF could act as a potent mitogen for melanocytes, whereas cell proliferation is independent of the presence of exogenously supplied HGF when melanocytes become malignant. Like HGF, SCF activated the proliferation of normal melanocytes, but no significant changes were obtained in melanoma cells [5]. Further, Natali et al. [13] reported that the loss of c-KIT expression was closely related to the malignant transformation of melanocytes, and Lassam and Bickford [10] observed loss of c-kit message in cultured human melanoma cells.

To date, however, no investigation has revealed the localisation and role of SCF during the stage of malignant transformation of pigment cells. Furthermore, as reported by Hibi et al. [8], SCF and c-KIT (c-Kit proto-oncogene product) were occasionally coexpressed in malignant cells at the protein and RNA level, suggesting that autocrine factors and their receptors could be implicated as regulatory mechanisms in some, but not all, tumour cells. Although genes encoding c-KIT and SCF were originally reported to play an important part in the regulation of mouse skin colour [14, 28], it is of interest to investigate their possible role during malignant transformation of human melanocytic lesions. The aim of the present study was therefore to compare the localisation

of SCF in normal skin, melanocytic naevi, and malignant melanomas and compare it with the distribution of its receptor, c-KIT. In addition, sodium butyrate and hexamethylene bisacetamide (HMBA), which have been reported to induce various phenotypic alterations in melanoma cells [18, 20], were applied to cultured human melanoma cells, to determine whether SCF and/or c-KIT could serve as markers for differentiation of pigment cells.

Materials and methods

One-hundred and twenty-six melanocytic tumours were chosen from the records of the Department of Dermatology, Sapporo Medical University School of Medicine, from 1981 to 1993. All pathologic tissues were removed surgically or biopsied, routinely fixed in 10% neutral formalin, and embedded in paraffin wax. Localisation of SCF and c-KIT in various components of normal skin were selected from an independent portion discontinous from the tumour (Table 1). Melanocytic lesions consisted of 69 cases of common melanocytic naevi (7 junctional types, 10 compound types, and 41 intradermal types), 2 cases of blue naevi, 5 cases of Spitz's naevi, and 4 cases of dysplastic naevi (Table 2). Thirty-five cases of primary melanomas (15 superficial spreading types, 7 nodular types, 8 lentigo maligna types, and 5 acral lentiginous types) and 22 cases of metastatic lesions were also studied to compare the results with those of benign counterparts (Table 2).

Procedures for cell culture have been described elsewhere [21]. All cultures were grown in RPMI 1640 medium supplemented with 5% fetal calf serum (FCS, v/v), penicillin (100 U/ml), streptomycin (100 μg/ml), and 3 mM 4-(2-hydroxyethyl)-1-piperazine ethanesulfate (HEPES) and were maintained at 37°C in a humidified atmosphere containing 5% CO_2. Melanoma cells used in the present study were MM96E [21], MM127 [25], MM138 [25], MM170 [25], and MM-S5 [16]. Furthermore, short-term culture of melanocytes from pigmented naevus (MC-SV1) were grown to compare the antigenic expression with that in malignant melanocytes by Western blot analysis.

Detailed procedures for immunohistochemistry have been reported elsewhere [22]. Briefly, serial sections were cut 4 μm thick from each specimen, and dewaxed with xylene and graded alcohols. Endogenous peroxidase activity was blocked with 0.3% H_2O_2 in methanol, and nonspecific reaction was reduced by incubation with normal goat serum. Polyclonal primary antibodies for human secretory-type SCF (clone K089) and c-KIT (clone K963) were obtained from IBL (Tokyo, Japan). After primary antibodies, diluted at 1:100 in PBS (pH 7.2), were applied overnight at 4°C, goat anti-rabbit immunoglobulins were reacted, followed by incubation with ABC-reagent (Nichirei, Tokyo, Japan). Immunoreaction was visualized with 3–3' diaminobenzidine (DAB), and sections were counterstained with Giemsa to differentiate immunoproducts from melanin pigment. All procedures were performed at room temperature (RT) unless otherwise stated.

In addition to the conventional procedures, trypsin digestion pretreatment and the microwave oven heating method reported by Shi et al. [17] were carried out to enhance the immunoreactivity of antibodies.

Western blot analysis was performed according to the methods reported by Towbin [23] and Burnette [3] with slight modifications and carried out at RT unless otherwise stated. Cells were harvested, rinsed in PBS (pH 7.2) and centrifuged. Cell pellets were resuspended in cell lysis buffer containing 10 mM Tris (pH, 7.4), 20% glycerol, 1% sodium dodecyl sulfate (SDS), and 2 mM phenylmethyl sulphonyl fluoride, and sonicated for 3 min. Cell lysates were then immersed in boiling water for 4 min and centrifuged at 10,000 rpm for 10 min. Aliquots (15–20 μl/lane) were loaded onto a 7.5% gel and electrophoresed in 25 mM Tris containing 192 mM glycine and 0.1% SDS. Molecular weight standards were purchased from Pharmacia LKB (Uppsala, Sweden). After transfer of proteins onto nitrocellulose membrane, immunostaining was carried out as described elsewhere [19]. In order to assess the potential of SCF and c-KIT as differentiation markers for melanocytes, human melanoma cells (MM96E and MM138) were cultured in the presence of sodium butyrate (1.5 mM) or HMBA (10 mM), since these doses were the most effective for induction of various phenotypic alterations in these cell lines with minimal cellular damage [18, 20]. After incubation with each drug for 48 h, cells were harvested with trypsin, washed in PBS (pH 7.2), and subjected to Western blot analysis.

Results

Localisation of SCF and c-KIT in tissues was studied using normal skin on the margin of a lesion, and results are summarised in Table 1. In various cutaneous components, localisation of SCF was observed predominantly in the endothelial cells of blood vessels (Fig. 1a), suggesting that these cells could be a source of the peptide to various cell types including melanocytes. This observation was recently confirmed by the study of Rochelle et al. [15]. Most of the other cell types in skin, however, failed to show SCF localisation, except in some cells such as fibroblasts and cells in peripheral nerves (Fig. 1b). Few basal melanocytes expressed SCF (not shown).

c-KIT generally tended to localise weakly in keratinocytes but most intensely in tissue mast cells (Fig. 1c), as was identifiable with Giemsa staining (not shown). Like SCF, a small proportion of normal melanocytes located in the basal layer of epidermis and hair follicles (Fig. 1c, d) were reactive with anti-c-KIT antibody, and these findings were consistent with those reported by others [24]. Again, most other cutaneous components failed to show significant c-KIT localisation.

In total, 126 tumours of melanocytic origin were subjected to immunohistochemical study (Table 2). To obtain a view on the significance of the data, we considered

Table 1 Expression of SCF and c-KIT in various components of normal skin (*SCF* stem cell factor, *c-KIT* c-Kit proto-oncogene product, *Kc* keratinocyte, *Mc* melanocyte)

Components	*n*	Number of positive cases	
		SCF	c-KIT
Epidermis			
Keratinocyte	26	1	15
Melanocyte	26	4	3
Langerhans cell	26	0	0
Dermis			
Sweat gland	12	0	0
Hair follicle; Kc	10	3	1
Hair follicle; Mc	10	0	2
Sebaceous cell	18	0	0
Fibroblast	25	7	1
Endothelial cell	25	20	0
Tissue mast cell	27	0	23
Peripheral nerve	15	8	0
Arrector pili muscle	10	0	0

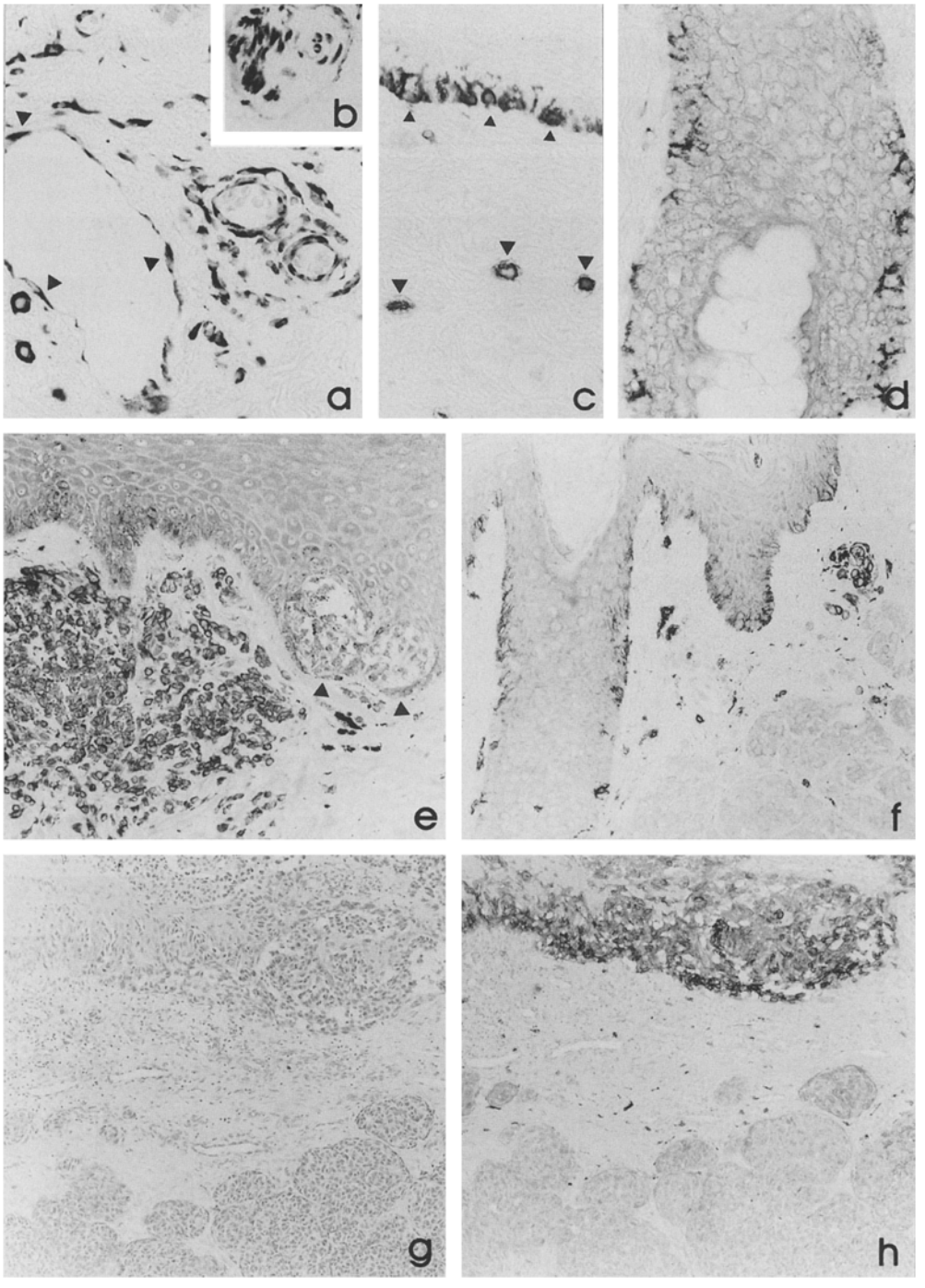

Fig. 1a–h Immunoproduct of anti-SCF antibody is observed in various cell types, such as endothelial cells of blood vessels (*arrowheads*) and fibroblasts (**a**), composing cells of peripheral nerve (**b**), and other dermal components. ×400. On the other hand, localisation of c-KIT is mainly observed in tissue mast cells (*large arrowheads*), basal melanocytes (*small arrowheads*) (**c**), and follicular melanocytes (**d**). ×400. Strong immunoreactivity of anti-SCF antibody is observed in dermal naevus cells, whereas that in epidermal melanocytic nests (*arrowheads*) is minimal or undetectable (**e**). ×180. Unlike SCF, c-KIT is predominantly found localised in epidermal melanocytic cells, while no immunoproducts are demonstrated in dermal naevus cells except in tissue mast cells (**f**). ×180. No immunoreaction of anti-SCF antibody is seen in either epidermal or dermal melanoma cells (**g**), whereas intense reaction of c-KIT is found in epidermal, but not in dermal melanoma cells, although the number of c-KIT positive cases was smaller than that of benign naevi (**h**). ×180

the results from two viewpoints: the number of cases showing positive staining (positive%) and the mean proportion of antibody-reactive cells calculated in four high power fields (reactive%).

SCF, the ligand for c-KIT, was observed in neoplastic melanocytes of 69 benign melanocytic lesions predominantly localised in dermal cells (Fig. 1e). As summarised in Table 2, 7 of 25 lesions with an epidermal melanocytic component (28%) showed a cytoplasmic reaction with anti-SCF antibody, while 51 of 62 cases of the dermal melanocytic component (82%) demonstrated distinct cytoplasmic reactivity. Reactive% of SCF-positive cells in epidermis and dermis was 28.6% (standard error of the mean, SE, 5.1%) and 62.2% (SE, 3.9%), respectively, a difference that was statistically significant as calculated by the Chi-square test ($P<0.0001$). In 35 cases of primary melanoma lesions, the percentage of SCF-expressing lesions (positive%) was significantly lower than that in melanocytic naevi (14%). No positive cells were found in 35 epidermal lesions, whereas 5 of 29 dermal lesions (17%) showed immunoreactivity. The reactive% ranged up to approximately 20% of tumour cells (mean±SE,

Table 2 Expression of SCF and c-KIT in benign and malignant melanocytic tumours

Type of tumour	*n*	Expression of SCF		Expression of c-KIT	
		No. of positive cases (positive %)	Range of reactive tumour cells (mean reactive %)	No. of positive cases (positive %)	Range of reactive tumour cells (mean reactive %)
Benign					
Common naevi:					
Junctional type	7	3 (42.9)	0–90 (34.2)	6 (85.7)	0–80 (45.7)
Compound type:	10				
Epidermal cell		3 (30.0)	0–90 (25.0)	7 (70.0)	0–80 (46.0)
Dermal cell		8 (80.0)	0–80 (71.0)	1 (10.0)	0–60 (6.0)
Intradermal type	41	40 (97.6)	0–90 (69.1)	0 (0)	0 (0)
Blue naevi	2	0 (0)	0 (0)	0 (0)	0 (0)
Spitz's naevi:	5				
Epidermal cell		0 (0)	0 (0)	1 (20.0)	0–40 (8.0)
Dermal cell		3 (60.0)	0–80 (34.0)	0 (0)	0 (0)
Dysplastic naevi:	4				
Epidermal cell		1 (25)	0–80 (35.0)	2 (50.0)	0–60 (20.0)
Dermal cell		1 (25)	0–80 (26.0)	0 (0)	0 (0)
Malignant					
Primary melanomas[a]	35	5 (14.3)		8 (22.9)	
Epidermal cell	35	0 (0)	0 (0)	8 (22.9)	0–80 (4.8)
Dermal cell	29	5 (17.2)	0–20 (2.0)	0 (0)	0 (0)
Metastatic melanomas	22	6 (27.3)	0–40 (4.6)	1 (4.5)	0–10 (0.5)

[a] Primary lesions were made up of 15 cases of superficial spreading type, 7 cases of nodular type, 8 cases of lentigo maligna type, and 5 cases of acral lentiginous type

2.0±0.4%; Fig. 1g). More metastatic lesions are SCF-positive (27%), but the reactive% (mean±SE, 4.6±1.8%) was only slightly higher than that observed in primary lesions. No significant difference was seen by statistical analysis between primary and metastatic lesions, possibly because of the small number tested.

Compared with the predominant dermal localisation of SCF, 16 of 69 benign melanocytic tumours (23%) demonstrated an epidermal c-KIT localisation (Fig. 1f). The immunoproduct was mostly restricted to the cytoplasm. The proportion of c-KIT-expressing cells varied from 0 to 80% (mean±SE, 36.1±6.3%), whereas that of dermal naevus cells was 0–60% (mean±SEM, 1.0±0.1%). However, though the number of melanoma lesions tested was small, 8 of 35 primary lesions (23%) and 1 of 22 metastatic lesions (4.5%) showed a distinct cytoplasmic localisation of c-KIT (Fig. 1h). Nonetheless, the proportion of c-KIT-positive tumour cells (reactive%) was significantly smaller (mean±SE, 4.8±0.6%) than that seen in the benign counterparts (mean±SE, 34.2±6.2%; $P<0.001$). Furthermore, the reactive% of cells expressing c-KIT was also lower in metastatic lesions (mean±SE, 0.5±0.1%), suggesting that the expression of c-KIT decreased with progressing malignant transformation of melanocytic cells, although its expression was generally low in benign tumours. We further stained a small number of frozen tissues to see whether there was a difference between formalin-fixed and frozen sections, finding no significant difference with respect to the reactivity of anti-SCF and c-KIT antibodies (not shown).

In addition, we performed two different pretreatments to see whether epitopes on SCF and c-KIT were masked by certain proteins. However, neither enzymatic digestion with trypsin nor microwave heating pretreatment [17] increased the immunoreactivity of antibodies against SCF and c-KIT (not shown).

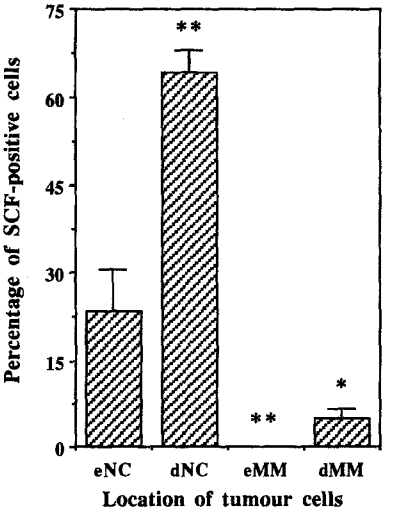

Fig. 2 Mean proportion of SCF-positive cells (reactive%) in each location. *Bar* standard error of the mean; *$P<0.001$; and **$P<0.0001$ (*e* epidermal, *d* dermal, *NC* naevus cell, *MM* melanoma cell)

The overall trend of the localisation of SCF is summarized in Fig. 2. Although the number of lesions tested might be small for the statistical analysis, significant differences in the reactive% were found when epidermal naevus cells were compared with dermal naevus cells and melanoma cells in each location.

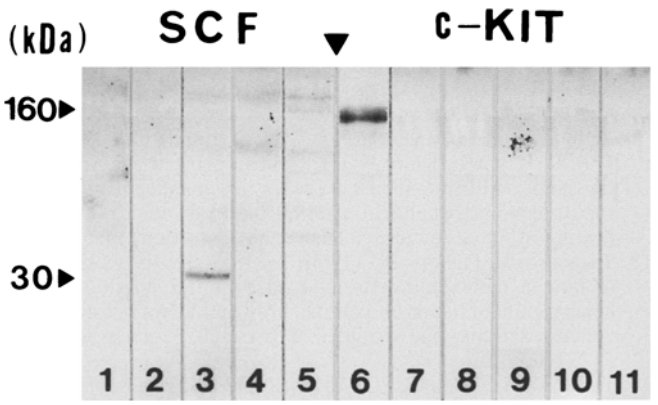

Fig. 3 Western blot analysis shows that the expression of SCF (lanes *1–5*) was observed only in MM138 cell extracts at approximately 30 kDa. None of melanoma cell lines (lanes *7–11*) demonstrated a band recognized by anti-c-KIT antibody, whereas MC-SV1 cells (lane *6*) showed a distinct band at approximately 160 kDa, a slightly higher molecular weight than reported previously [27]. *Lanes 1, 7* MM96E, *2, 8* MM127, *3, 9* MM138, *4, 10* MM170, *5, 11* MM-S5, *6* MC-SV1

In order to investigate qualitative and quantitative difference of SCF and c-KIT further at the protein level, we carried out Western blot and immunostaining under non-reducing conditions. Of the five melanoma cell lines used, SCF-expression was observed in only one (MM138) at a molecular weight of approximately 30 kDa (Fig. 3); that previously reported was 32 kDa [1]. Furthermore, all melanoma cell lines failed to show an appropriate band of c-KIT, although MC-SV1 revealed a distinct band at approximately 160 kDa (Fig. 3), a slightly higher molecular weight than reported previously (145 kDa) [27].

We also used two phenotypic modifiers to evaluate the phenotypic expression of the antigens in melanoma cells (MM96E and MM138). In contrast to suppression of c-MET [16], treatment with sodium butyrate and HMBA failed to induce any alteration of either the qualitative or quantitative levels of SCF in two melanoma cell lines as judged by Western blot analysis (not shown).

Discussion

A number of complicated mechanisms are involved in malignant transformation of various cell types in either a specific or a general manner, including oncogene and proto-oncogene changes. However, there may be differences between benign and malignant cells in the effects induced by these regulators, ligands and receptors. For example, recently-reported growth factors for melanocytes, HGF and SCF, activate the proliferation of normal melanocytes only as long as they retain a benign phenotype. Natali et al. [13] reported a progressive loss of c-KIT expression according to a malignant transformation of melanocytes, and Lassam and Bickford [10] demonstrated that the c-Kit message was lost in cultured melanoma cells.

In the present study we compared the localisation of SCF and its receptor c-KIT at the histological level to clarify the possible role of this ligand/receptor combination during melanomagenesis. The results can be summarised as follows. (1) In normal skin, localisation of SCF was observed predominantly in endothelial cells of blood vessels, whereas tissue mast cells constantly expressed c-KIT, which can function as a receptor for MGF. (2) Localisation of SCF and c-KIT in benign neoplastic melanocytes was rather seen in a complementary fashion; reactivity of anti-SCF antibody was almost always found in dermal naevus cells, while that of anti-c-KIT antibody was recognised mainly in epidermal melanocytic cells, although the expression of the latter protein was generally found in a small proportion of specimens. (3) The proportion of anti-SCF and c-KIT-positive cells (reactive%), but not the number of positive cases (positive%), was significantly smaller in melanoma cells than in cells from benign counterparts. (4) On Western blot analysis, only one melanoma cell line (MM138) showed a distinct band of SCF at approximately 30 kDa, and no melanoma cell line expressed c-KIT. (5) No evidence of the coexpression of SCF and c-KIT in tumour cells reported in small cell lung cancer lines [8] was obtained in melanocytic tumours. (6) No significant changes were observed in MM96E and MM138 melanoma cells after application of phenotypic modifiers, suggesting that SCF cannot be regarded as a marker for melanocyte differentiation.

As demonstrated in the present study, secretory-type SCF was localised predominantly in endothelial cells in normal cutaneous components and in dermal naevus cells, but not in normal basal melanocytes. Murphy et al. [12] reported that melanocyte precursors require SCF for survival but not for cell differentiation, in keeping with the observation that dermal naevus cells tend to be biologically inactive but not differentiated, judging by the presence of immature melanosomes and low tyrosinase content [9]. The obverse localisation of c-KIT in epidermal naevus cells suggests that the growth factor/ligand association in melanocytes is disturbed in neoplastic cells on a site specific basis.

Loss of SCF may represent a part of the escape from autonomous growth regulation but not from cellular differentiation. Loss of the receptor for SCF, c-KIT, was less frequent than for SCF, and therefore may not have a critical role in malignant transformation. These data are partly consistent with those reported by Natali et al. [13], and could be interpreted as showing that SCF in neoplastic melanocytes plays a critical part in the malignant transformation of pigment cells, particularly with respect to the cellular proliferation.

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References

1. Anderson DM, Lyman, SD, Baird A, Wignall JM, Eisenman J, Rauch C, March CJ, Boswell HS, Gimpel SD, Cosman D,

Williams DD (1990) Molecular cloning of mast cell growth factor, a hematopoietin that is active in both membrane bound and soluble forms. Cell 63: 235–243
2. Becker D, Meier CB, Herlyn M (1989) Proliferation of human malignant melanomas is inhibited by anti sense oligodeoxynucleotides targeted against basic fibroblast growth factor. EMBO J 8: 3685–3691
3. Burnette WN (1981) "Western blotting": electrophoretic transfer of proteins from sodium dodecyl sulfate-polyacrylamide gels to unmodified nitrocellulose and radiographic detection with antibody and radioiodinated protein A. Anal Biochem 112: 195–203
4. Cantley LC, Auger KR, Carpenter C, Duckworth B, Graziani A, Kapeller R, Soltoff S (1991) Oncogenes and signal transduction. Cell 64: 281–302
5. Funasaka Y, Boulton T, Cobb M, Yarden Y, Fan B, Lyman SD, Williams DE, Anderson DM, Zakut AR, Mishima Y, Halaban R (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
6. Halaban R, Ghosh S, Baird A (1987) FGF is a putative natural growth factor for human melanocytes. In Vitro Cell Dev Biol 23: 47–52
7. Halaban R, Rubin JS, Funasaka Y, Cobb M, Boulton T, Faletto D, Rosen E, Chan A, Yoko K, White W, Cook C, Moellmann G (1992) Met and hepatocyte growth factor/scatter factor signal transduction in normal melanocytes and melanoma cells. Oncogene 7: 2195–2206
8. Hibi K, Takahashi T, Sekido Y, Ueda R, Hida T, Ariyoshi Y, Takagi H, Takahashi T (1991) Coexpression of the stem cell factor and the c-kit genes in small-cell lung cancer. Oncogene 6: 2291–2296
9. Jimbow K, Quevedo WC, Fitzpatrick TB (1976) Some aspects of melanin biology: 1950–1975. J Invest Dermatol 67: 72–89
10. Lassam N, Bickford S (1992) Loss of c-kit expression in cultured melanoma cells. Oncogene 7: 51–56
11. Matsumoto K, Tajima H, Nakamura T (1991) Hepatocyte growth factor is a potent stimulator of human melanocyte DNA synthesis and growth. Biochem Biophys Res Commun 276: 45–51
12. Murphy M, Reid K, Williams DE, Lyman SD, Bartlett PF (1992) Steel factor is required for maintenance, but not differentiation, of melanocyte precursors in the neural crest. Dev Biol 153: 396–401
13. Natali PG, Nicotra MR, Winkler AB, Cavalier AB, Ullrich A (1992) Progression of human cutaneous melanoma is associated with loss of expression of c-kit proto-oncogene receptor. Int J Cancer 5: 197–201
14. Nocka K, Majumder S, Chabot B, Ray P, Cervone M, Bernstein A, Besmer P (1989) Expression of c-kit gene products in known cellular targets of W mutations in normal and W mutant mice – evidence for an impaired c-kit kinase in mutant mice. Genes Dev 3: 816–826
15. Rochelle R, Whitaker-Menezes D, Longley J, Murphy GF (1995) Human dermal endothelial cells express membrane-associated mast cell growth factor. J Invest Dermatol 104: 101–106
16. Saitoh K, Takahashi H, Sawada N, Parsons PG (1994) Detection of the c-met proto-oncogene products in normal skin and tumours of melanocytic origin. J Pathol (Lond) 174: 191–199
17. Shi SR, Key ME, Kalra KL (1991) Antigen retrieval in formalin-fixed, paraffin-embedded tissues: an enhancement method for immunohistochemical staining based on microwave oven heating of tissue sections. J Histochem Cytochem 39: 741–748
18. Takahashi H, Parsons PG (1990) In vitro phenotypic alteration of human melanoma cells induced by differentiating agents: heterogenous effects on cellular growth and morphology, enzymatic activity, and antigenic expression. Pigment Cell Res 3: 223–232
19. Takahashi H, Parsons PG (1992) Rapid and reversible inhibition of tyrosinase activity by glucosidase inhibitors in human melanoma cells. J Invest Dermatol 98: 481–487
20. Takahashi H, Saitoh K (1994) Phenotypic modifier hexamethylene bisacetamide (HMBA) induces various phenotypic alterations in human melanoma cells (in Japanese). Jpn J Dermatol 104: 839–845
21. Takahashi H, Schumann R, Quinn R, Briscoe TA, Parsons PG (1991) Isomers of a marine diterpene distinguish sublines of human melanoma cells on the basis of apoptosis, cell cycle arrest and differentiation markers. Melanoma Res 1: 359–366
22. Takahashi H, Strutton GM, Parsons PG (1991) Determination of proliferating fractions in malignant melanomas by anti-PCNA/cyclin monoclonal antibody. Histopathology 18: 221–227
23. Towbin H, Staehelin T, Gordon J (1979) Electrophoretic transfer of proteins from polyacrylamide gels nitrocellulose sheets: procedure and some applications. Proc Natl Acad Sci USA 76: 4359–4354
24. Tsuura Y, Hiraki H, Watanabe K, Igarashi S, Shimamura K, Fukuda T, Suzuki T, Seito T (1994) Preferential localization of c-kit product in tissue mast cells, basal cells of skin, epithelial cells of breast, small cell lung carcinoma and seminoma/dysgerminoma in human: immunohistochemical study on formalin-fixed, paraffin-embedded tissues. Virchows Arch 424: 135–141
25. Whitehead RH, Little JH (1973) Tissue culture studies on human malignant melanoma. Pigment Cell 1: 382–389
26. Yada Y, Higuchi K, Imokawa G (1991) Effects of endothelins on signal transduction and proliferation in human melanocytes. J Biol Chem 266: 18352–18357
27. Yarden Y, Kuang W-J, Yang-Feng T, Coussens L, Munemitsu S, Dull TJ, Chen E, Schlessinger J, Francke U, Ullrich A (1987) Human proto-oncogene c-kit: a new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J 6: 3341–3351
28. Zsebo K, Williams DA, Geissler EN, Broudy VC, Martin FH, Atkins HL, Hsu R-Y, Birkett KE, Smith KA, Takeishi T, Cattanach BM, Galli SJ, Suggs S (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