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Original Paper

Recombinant Human Stem Cell Factor Does Exert Minor Stimulation of Growth in Small Cell Lung Cancer and Melanoma Cell Lines

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We have previously reported on the stimulation of clonal growth of a glioblastoma cell line by rhSCF (Berdel *et al.*, *Cancer Res* 1992, 52, 3498–3502). Within an extensive screening programme of haematopoietic growth factor activity on malignant cells, the effects of rhSCF were further tested on the growth of 29 different human cell lines derived from a wide range of solid tumours, among them six lung cancers and five melanomas. RhSCF (0, 1, 10, 100 ng/ml) was tested in a human tumour cloning assay (HTCA) which reliably detects growth modulation of tumour cells by cytokines. Additionally, a tritiated thymidine uptake test was used. Growth of 27 of the 29 cell lines tested was not affected by rhSCF. However, growth of the small cell lung cancer (SCLC) cell line HTB 120 was slightly stimulated (1.5 fold that of controls), and that of the melanoma cell line MeWo was stimulated up to 1.3-fold. This activity was eliminated dose-dependently by the tyrosine kinase inhibitor, genistein. We further analysed the cell lines for expression of the proto-oncogene *C-KIT* and its ligand SCF. All melanoma and lung cancer cell lines expressed SCF as assessed at the mRNA level. Northern blotting also revealed clear *C-KIT* mRNA expression in three melanoma (HAS, MeWo, SK-MEL-28), one NSCLC (HTB 53), and four SCLC cell lines (HTB 119, HTB 120, HTB 171, HTB 175). Furthermore, *C-KIT* protein expression was detected by flow cytometric analysis on the cell surface of MeWo, HTB 119 and HTB 120 cells. Our data indicate that SCF can be operative in growth modulation of non-haematopoietic malignant cells, especially SCLC and melanoma. However, our extensive screening of SCF/tumour cell interaction shows that this interaction is rare and makes potential hazards, such as tumour stimulation upon clinical use of rhSCF in conjunction with chemotherapy in cancer patients, unlikely for the majority of other tumour histologies.

Key words: SCF and solid tumours, c-*kit*, small cell lung cancer, melanoma, growth modulation
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INTRODUCTION

RECENT MOLECULAR cloning of the stem cell factor (SCF) gene has revealed that SCF, also designated as mast cell growth factor (MGF), Steel locus factor (SLF), or kit ligand (KL) is a pluripotent costimulating cytokine for haematopoietic progenitor cells [1–10]. The receptor for SCF is encoded by the proto-oncogene *C-KIT* [11, 12] which represents the cellular homologue of v-*kit*, the Hardy–Zuckerman 4 feline sarcoma virus oncogene [13]. The *C-KIT* receptor protein has a split intracytoplasmic tyrosine kinase domain and is structurally very closely related to the *C-FMS* product, the receptor for macrophage colony-stimulating factor (M-CSF or CSF-1), and to the receptor for platelet-derived growth factor (PDGF) [11, 14; see ref. 15 for review]. Genetic studies have demonstrated that *C-KIT* is allelic to the murine white spotting (*W*) locus mapped to the chromosome 5, thus indicating that *C-KIT* plays an important role during haematopoiesis and gametogenesis, as well as during melanogenesis [12, 16–18].

SCF has been shown to be the product of the Steel (*SI*) locus on mouse chromosome 10. Mutations at the *SI* locus result in phenotypic characteristics that are very similar to those seen in mice carrying *W* mutations, i.e. they affect haematopoiesis, gametogenesis and melanogenesis [19, 20]. *In vitro*, rhSCF alone or in combination with other growth factors is able to increase both the number and size of the haematopoietic colonies [7, 10, 21, 22], to stimulate the growth of mast cell colonies [4, 23], to support the proliferation of B-cell progenitors in conjunction with interleukin 7 (IL-7) [7], to stimulate growth

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and attachment of primordial germ cells [24], and to increase self-renewal and terminal divisions of leukaemic blasts [25].

C-KIT is expressed by cells from normal bone marrow and normal breast epithelium, by glial cells both in the brain and in the Auerbach's and Meissner's plexuses of the gut, as well as by normal adrenal medullary cells, mast cells and melanocytes [3, 4, 26–30]. Different human malignancies have also been shown to express *C-KIT*, among them leukaemias [31], glioblastomas [32, 33], small cell lung cancers [34, 35], testicular germ tumours [36], gynaecological tumours (ovarian and cervical cancers) [37] and melanomas [30]. The SCF gene is abundantly expressed in a broad spectrum of human cancers [34]. Coexpression of both the ligand and the receptor is seen only in very few types of cancer [30, 34, 35, 37], suggesting possible involvement of autocrine stimulation via this ligand–receptor system in the pathogenesis of these tumour entities. Our group is investigating the growth-modulating effects of haematopoietins, including SCF, in non-haematopoietic malignant cells [see ref. 38 for review]. Hence, we studied the effect of rhSCF on the proliferation and clonal growth of a wide variety of human malignant cell lines derived from solid tumours. Initial results of this study with a small number of cell lines have been previously presented [39, 40].

MATERIALS AND METHODS

Cell lines

Cell lines derived from human non-haematopoietic tumours were purchased from the American Type Culture Collection (ATCC) (Rockville, Maryland, U.S.A.), with the following exceptions: "B" is a prostate carcinoma cell line originating from our own laboratory, and the melanoma cell lines were a kind gift from Dr C. Garbe (Department of Dermatology, Universitaetsklinikum Benjamin Franklin). Cell culture techniques have been described elsewhere in detail [41].

Cytokines and antibodies

Recombinant human SCF was kindly provided by Amgen Inc. (Thousand Oaks, California, U.S.A., Lot 500–1). It was derived from *Escherichia coli*, its concentration was 1.33 mg/ml and its purity 95% by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis. The biological activity of the material was confirmed by colony stimulation of the glioblastoma cell line CRL 1620 [33]. Neutralising sheep polyclonal antibody against hSCF was purchased from Genzyme (Cambridge, Massachusetts, U.S.A.). The antibody YB5.B8 used was a kind gift from Dr K.L. Ashman (University of Adelaide, Adelaide, Australia). It is a mouse monoclonal anti-*C-KIT* receptor antibody of immunoglobulin G (IgG) class. Details on production and characterisation of its biological activity have been described elsewhere [26, 31]. The monoclonal antibody SR-1 (IgG2a) was kindly provided by Dr V. Broudy (University of Washington, Seattle, Washington, U.S.A.). This antibody recognises an epitope on the *C-KIT* receptor different from that recognised by YB5.B8 [42].

Human tumour cloning assay (HTCA)

Cells were tested in the HTCA modification, yielding optimal clonal growth for the respective cell line. The HTCA using agar containing capillaries ($HTCA_{cap}$ [43]) was performed in a modification previously described [33] with the cell lines "B", and HTB 85. For evaluation of clonal growth of the cell lines HTB 26, HTB 119 and HTB 120, a methylcellulose assay ($HTCA_{mc}$) was used as previously published in detail [38]. All other cell lines were tested in a newly developed HTCA using mixtures of methylcellulose and agar ($HTCA_{mix}$) [44]. Cells were washed with their own medium, resuspended with 1 ml RPMI 1640 (Gibco, Glasgow, U.K.) plus 10% FCS and counted by trypan blue staining before added to the assay. The final incubation mixture of the HTCA contained the final growth factor concentrations as indicated in the tables, so the cells were exposed to SCF for the complete assay period. The numbers of cells seeded were 4×10^3 per capillary in the $HTCA_{cap}$ and 1×10^3 per dish in the other HTCA modifications. The colony formation was evaluated with an inverted microscope before and after an incubation period of 10 days at pH 7.2, 37°C, 5% CO_2 and high humidity.

We also tested the effects of the tyrosine kinase inhibitor, genistein (Sigma), on rhSCF-stimulated clonal growth of the human melanoma cell line MeWo. In brief, MeWo cells were precultured with genistein at various concentrations (range 0.37–3.7 μg/ml; see Figure 1) for 1 h. Thereafter, cells were washed, resuspended in fresh medium and assayed in a $HTCA_{mix}$ with rhSCF at a final concentration of 10 ng/ml. All incubations with rhSCF were performed at a concentration of 10 ng/ml.

Tritiated thymidine uptake

For this assay, 100 μl aliquots of rhSCF in "test medium" (usual growth medium of the tested cell lines) were seeded into six wells per test group of 96-well flat-bottomed microtitre plates (Greiner, Nuertingen, Germany) in concentrations as indicated. 100 μl of the cell suspension containing 5×10^4 cells (concentration per well) were added to the "test medium". Controls contained 100 μl of pure "test medium" instead of growth factor. Plates were incubated at 37°C, pH 7.2, in 5% CO_2 and high humidity for 72 h. The cultures were pulsed for the last 48 h with 0.5 μCi of [^{3}H]thymidine [specific activity 5.0 Ci/

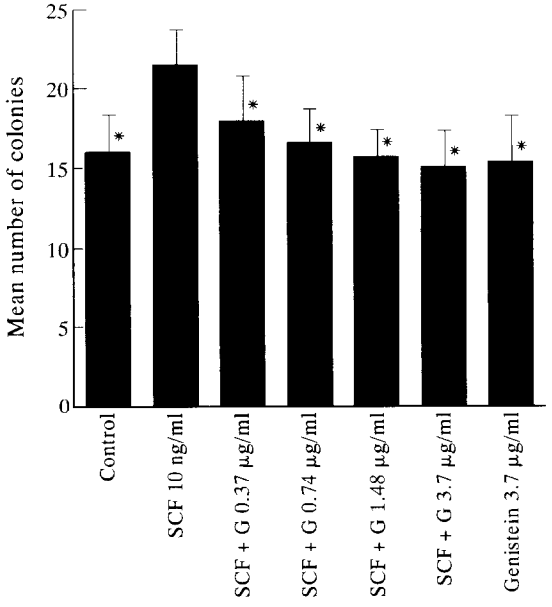

Figure 1. Effects of the tyrosine kinase inhibitor genistein (G) on rhSCF-stimulated clonal growth of the human melanoma cell line MeWo *in vitro*. All incubations with rhSCF were performed at a concentration of 10 ng/ml. Control represents tumour cells without cytokine or genistein. Values are means (± S.D.) of six assays. * *P* value < 0.05 when compared with SCF alone at 10 ng/ml (Mann–Whitney test). Control and genistein 3.7 μg/ml were not significantly different.

mmol.; Amersham and Buchler, Braunschweig, Germany) per well. The samples were processed, harvested, and counted in an LKB Betaplate system (LKB; Pharmacia, Freiburg, Germany). Values are means ± S.D.

Reverse-transcription polymerase chain reaction (RT-PCR)

Total RNA from HTB 119, which has been previously described to have multiple copies of SCF mRNA [45], was extracted according to the method of Chomczynski and Sacchi [46]. First-strand cDNA was synthesised from total RNA (1 μg) by 1 h incubation at 37°C with Maloney murine leukaemia virus (M-MLV) reverse transcriptase (Gibco BRL) and oligo(dT) priming. cDNA was then amplified by PCR using 2.5 U *Thermus aquaticus (Taq)* DNA polymerase (Perkin–Elmer Cetus, Norwalk, Connecticut), 50 pmol of a 25-nucleotide sense primer specific for nucleotides 512–536 (5'-AC CTT GTG GAG TGC GTG AAA GAA AA-3') of the published SCF cDNA sequence [7], and 50 pmol of a 25-nucleotide antisense primer complementary to nucleotides 902–926 (5'-AC TGC CCT TGT AAG ACT TGG CTG TC-3') (TIB MolBiol, Berlin, Germany). Reactions were performed in a DNA thermocycler (Perkin–Elmer Cetus) and the cycling parameters were 30 cycles of 1 min of denaturation at 94°C, 2 min of annealing at 55°C, and 2 min of synthesis at 72°C. The length of the PCR product was 415 bp. Identification of this DNA fragment was made by automated DNA sequencing on an ABI 373A DNA sequencer using *Taq* DNA polymerase and dye-terminator technology.

Northern blot analysis

Total cellular RNA was isolated from cells according to the method described above. Subsequently, 15 μg of isolated RNA of every cell sample was electrophoresed in a 1% agarose gel containing 2.4% formaldehyde and then transferred by vacuum in 10× standard saline citrate (SSC) to a Nylon membrane (Hybond-N membrane; Amersham, U.K.). The blots were baked for 2 h at 80°C. After prehybridisation for 3 h at 42°C in 5× Denhardt's solution, 50% formamide, 1% SDS, 1 mol/l NaCl, 50 mmol/l piperazine-*N*,*N*'-bis (2-ethanesulphonic acid), 10 mmol/l EDTA, and 100 μg/ml herring sperm DNA, the blots were exposed to an overnight hybridisation (using the same solution as for prehybridisation with the addition of 0.3 mol/l NaCl) with a cDNA probe for SCF (produced from HTB 119 by RT-PCR) or an oligonucleotide probe for *C-KIT* (Oncogene Science, New York, U.S.A., Lot 8940–1). The SCF-cDNA was previously radiolabelled with [α-^{32}P]dCTP using a megaprime DNA labelling system (Amersham). For labelling the oligonucleotide probe, a terminal transferase DNA-tailing kit (Boehringer Mannheim, Germany) was used. The blots were then washed twice in 2 × SSC: 1% SDS at 60°C. Finally, the blots were autoradiographed for 18 h with a Kodak X/Omatic film (Eastman Kodak, Rochester, New York, U.S.A.) at −80°C. The blots were checked for integrity and comparable RNA loading by reprobing with an antisense α-glyceraldehyde-3-phosphate dehydrogenase (α-GAPDH) (Oncogene) or a β-actin (Dianova-Immunotech, Hamburg, Germany) specific oligonucleotide.

Flow cytometry

Cells were freshly detached from plastic tissue culture flasks (when grown as monolayer) by exposure at 4°C to 0.6 mM EDTA in phosphate-buffered saline (PBS) and then washed with PBS containing 5% fetal calf serum (FCS) and 0.1% NaN_3. Subsequently, 1 × 10^6 cells were treated with saturating concentrations of YB5.B8 or SR-1 (1:1000 ascites dilution) monoclonal anti-*C-KIT* antibodies at 4°C for 30 min. Cells were then washed with PBS/FCS/NaN_3 to remove unbound antibody and incubated with an appropriate dilution of phycoerythrin (PE)-conjugated $F(ab)_2$ goat-anti-mouse IgG (Dianova) at 4°C for 30 min. Controls were incubated with an isotype-matched irrelevant monoclonal antibody and the same second antibody. After two washes with PBS/0.1% bovine serum albumin (BSA)/ 0.1% NaN_3 cells were analysed by using a FACScan (fluorescence-activated cell sorter) analyser equipped with LYSYS II software (Becton-Dickinson, Heidelberg, Germany).

Statistics

The significance of difference at each experimental condition (control, each single concentration of rhSCF) was calculated by a non-parametric test (Mann–Whitney; Kruskal–Wallis test as indicated). P values < 0.05 were interpreted as indicating significant differences.

RESULTS

Effects of rhSCF

The effects of rhSCF were tested on the growth of 29 different cell lines derived from a wide range of solid tumours. The histologies of the cell lines tested are outlined below and in the tables. We cultured the cell lines in the presence of increasing concentrations (1, 10, 100 ng/ml) of rhSCF or without rhSCF (control) and assayed the influence of the cytokine on the growth of these cell lines using a HTCA. The rhSCF preparation used was tested for biological activity throughout the study. This activity was confirmed by colony stimulation in a $HTCA_{mix}$ of the glioblastoma cell line CRL 1620, which has previously been shown to be sensitive for rhSCF in a $HTCA_{cap}$ [33]. The response to the cytokine was completely reversible by pre-incubating rhSCF with a neutralising sheep polyclonal antibody against hSCF, confirming specificity of the observation (data not shown in detail).

rhSCF did not show major (< 0.5-fold or > 1.5-fold of the controls), significant (Kruskal–Wallis test) and reproducible modulation of the growth of the following malignant cell lines: breast (HTB 22, HTB 26), choriocarcinoma (HTB 36), colon (HTB 38), gastric (KATO III), head and neck (HTB 43, CCL 23, CCL 17), kidney (HTB 46), liver (HepG2, H-5), neuroblastoma (CCL 127), osteosarcoma (CRL 1427, HTB 85), ovarian (HTB 77), pancreatic (MiaPaCa), prostate ("B"), and rhabdomyosarcoma (HTB 82). Only marginal stimulation of the H-5 liver carcinoma cell line (1 ng/ml rhSCF: 116% of controls, $P = 0.0465$) and the HTB 38 cell line (10 ng/ml rhSCF: 111% of controls, $P = 0.0139$) was observed.

Tables 1 and 2 summarise the clonal growth of lung cancer and melanoma cell lines with and without rhSCF. Growth of the small cell lung cancer (SCLC) cell line HTB 120 (NCI-H 128) was significantly and reproducibly stimulated up to 1.5-fold by rhSCF at 10 ng/ml ($P = 0.0004$). Higher cytokine concentrations than 10 ng/ml resulted in saturation of the effect in the HTCA. Statistical evaluation of the results with the melanoma cell line MeWo showed a significantly higher number of colonies in the SCF-incubated cultures than in the controls ($P = 0.0042$), however, growth stimulation was only up to 1.3-fold and thus minor.

Additionally, a tritiated thymidine uptake assay was used. This assay uses a higher cell density than the HTCA and cells are allowed to adhere during the assay period. In the tritiated thymidine uptake assay, growth stimulation of the HTB 120

Table 1. Effects of rhSCF on colony formation (HTCA) of human lung carcinoma cell lines in vitro

Cell line	Assay	Controls		Colonies (% of controls) with rhSCF at			n†	P value‡
		No. colonies/dish*	% colonies	1 ng/ml	10 ng/ml	100 ng/ml		
Small cell lung cancer								
HTB 119	$HTCA_{mc}$	35.5 ± 4.9	100 ± 13.8	96.6 ± 15.2	96.3 ± 12.7	103.7 ± 9.6	6	0.4463
HTB 120	$HTCA_{mc}$	12.5 ± 1.6 9.2 ± 3.1	100 ± 24.1	100.8 ± 30.1	151.5 ± 21.6	127.4 ± 40.1	12	0.0004
HTB 171	$HTCA_{mix}$	88.5 ± 15.4	100 ± 17.4	96.4 ± 13.2	96.8 ± 24.1	97.2 ± 18.2	6	0.9902
HTB 175	$HTCA_{mix}$	150.7 ± 13.2	100 ± 8.8	95.1 ± 7.3	96.4 ± 8.6	99.7 ± 6.2	6	0.6106
Non-small cell lung cancer								
HTB 53	$HTCA_{mix}$	149.5 ± 15.3	100 ± 10.2	97.1 ± 10.6	95.2 ± 10.0	95.9 ± 6.0	6	0.7695
HTB 56	$HTCA_{mix}$	19.2 ± 3.3	100 ± 17.2	84.9 ± 8.3	92.7 ± 10.9	96.4 ± 15.1	6	0.3881

* Mean ± S.D. of absolute numbers of colonies/dish. Values are given for each single experiment and represent means of 6-fold assays. † Number of dishes counted at each single condition (0, 1, 10, 100 ng/ml of rhSCF). ‡ *P* values determined by Kruskal–Wallis test.

Table 2. Effects of rhSCF on colony formation (HTCA) of human melanoma cell lines in vitro

Cell line	Assay	Controls		Colonies (% of controls) with rhSCF at			n†	P value‡
		No. colonies/dish*	% colonies	1 ng/ml	10 ng/ml	100 ng/ml		
A 375	$HTCA_{mix}$	168.3 ± 12.8	100 ± 7.6	94.3 ± 6.2	97.6 ± 9.8	89.8 ± 5.2	6	0.1199
HAS	$HTCA_{mix}$	39.5 ± 9.8	100 ± 24.8	104.6 ± 19.5	105.1 ± 12.7	101.3 ± 22.5	6	0.9912
MeWo	$HTCA_{mix}$	53.8 ± 14.7 15.5 ± 3.8 40.3 ± 5.4	100 ± 21.1	102.5 ± 24.4	120.6 ± 24.5	122.2 ± 24.5	18	0.0042
SK-MEL-28	$HTCA_{mix}$	21.7 ± 3.1	100 ± 14.2	94.5 ± 17.0	116.6 ± 11.1	100.5 ± 16.6	6	0.0832
ST-MEL-11	$HTCA_{mix}$	46.7 ± 5.5	100 ± 11.8	99.1 ± 15.0	106.3 ± 10.3	94.6 ± 9.4	6	0.7964

* Mean ± S.D. of absolute numbers of colonies/dish. Values are given for each single experiment and represent means of 6-fold assays. † Number of dishes counted at each single condition (0, 1, 10, 100 ng/ml of rhSCF). ‡ *P* values determined by Kruskal–Wallis test.

and MeWo cell lines was 1.4-fold ($P = 0.0022$) and 1.9-fold ($P = 0.0004$), respectively. There was a clear dose related enhancement of thymidine uptake induced by rhSCF at concentrations between 1 and 100 ng/ml (Table 3).

SCF-dependent growth stimulation of the cells was sensitive to the tyrosine kinase inhibitor, genistein. Treatment of these cells with genistein at non-toxic concentrations abrogated the stimulatory effect of rhSCF on colony formation in the HTCA (Figure 1).

Table 3. Effects of rhSCF on the growth of a melanoma (MeWo) and an SCLC cell line (HTB 120) in vitro *in two different assay systems*

Assay system	Controls		% of controls with rhSCF at			n†	P value‡
	Colonies/dish* or CPM	%	1 ng/ml	10 ng/ml	100 ng/ml		
HTCA colony formation							
MeWo ($HTCA_{mix}$)	53.8 ± 14.7 15.5 ± 3.8 40.3 ± 5.4	100 ± 21.1	102.5 ± 24.4	120.5 ± 24.5	122.2 ± 24.5	18	0.0042
HTB 120 ($HTCA_{mc}$)	12.5 ± 1.6 9.2 ± 3.1	100 ± 24.1	100.8 ± 30.1	151.5 ± 21.6	127.4 ± 40.1	12	0.0004
Tritiated thymidine uptake							
MeWo	12402 ± 2450§	100 ± 19.8	113.1 ± 22.1	176.4 ± 12.4	194.1 ± 15.6	6	0.0004
HTB 120	7379 ± 1019	100 ± 13.8	101.4 ± 14.1	124.1 ± 16.0	140.2 ± 12.1	6	0.0022

* Mean ± S.D. of absolute numbers of colonies/dish. Values are given for each single experiment and represent means of 6-fold assays. † Number of dishes counted at each single condition (0, 1, 10, 100 ng/ml of rhSCF). ‡ *P* values determined by Kruskal–Wallis test. § Mean ± S.D. of cpm.

SCF and C-KIT expression by lung cancer and melanoma cells

It has been demonstrated that SCF and *C-KIT* are frequently coexpressed in SCLC [34, 35]. In contrast, the progression of human melanomas has been associated with loss of expression of *C-KIT* receptor [47]. Therefore, we examined lung cancer and melanoma cell lines for expression of the *C-KIT* proto-oncogene and its ligand on the mRNA level. SCF mRNA was found in all SCLC and melanoma cell lines tested (Figure 2). Also, the ST-MEL-11, MeWo and SK-MEL-28 melanoma cell lines and the lung cancer cell lines HTB 119, HTB 120, HTB 171 and HTB 175 displayed *C-KIT* transcripts detectable in 15 μg RNA by Northern blot analysis (see Figure 2).

Flow cytometric analysis was performed with the cell lines to correlate the results obtained in the growth assay with the presence or absence of the *C-KIT* product at the protein level. FACS analysis showed the presence of this binding site for hSCF on a high percentage of the MeWo (Figure 3b) and HTB 120 cells (Figure 3e) cells. A lower expression of the *C-KIT* gene product was detected on the cell surface of the cell line HTB 119 (Figure 3d). There were no clear positive results for the other cell lines (see Figures 3c and 3f).

DISCUSSION

We studied the effect of rhSCF on the growth of 29 different human cell lines, including five melanomas, four SCLC and two non-small cell lung cancers (NSCLC). We selected HTCA for assaying anchorage-independent clonal tumour cell growth as the main test system of this investigation, since HTCA can reliably detect growth modulation of tumour cells by cytokines [44], and is predictive for *in vivo* tumorigenicity of xenotransplanted tumour cells and *in vivo* modulation of tumour growth by cytokines [44, 48, 49]. Additionally, a tritiated thymidine uptake assay was used, which involves a higher cell density than the HTCA and cells are allowed to adhere during the assay period. Growth of 27 of the 29 cell lines was not affected by rhSCF. However, growth of the SCLC cell line HTB 120 was significantly stimulated, as was the melanoma cell line MeWo. All melanomas and SCLC lines expressed SCF at the mRNA level, and *C-KIT* mRNA expression was observed in three melanoma and in all SCLC cell lines tested. Furthermore, flow cytometric analysis revealed *C-KIT* expression on the surface of a melanoma (MeWo) and two SCLC cell lines (HTB 119 and HTB 120). Interestingly, there was no complete correlation

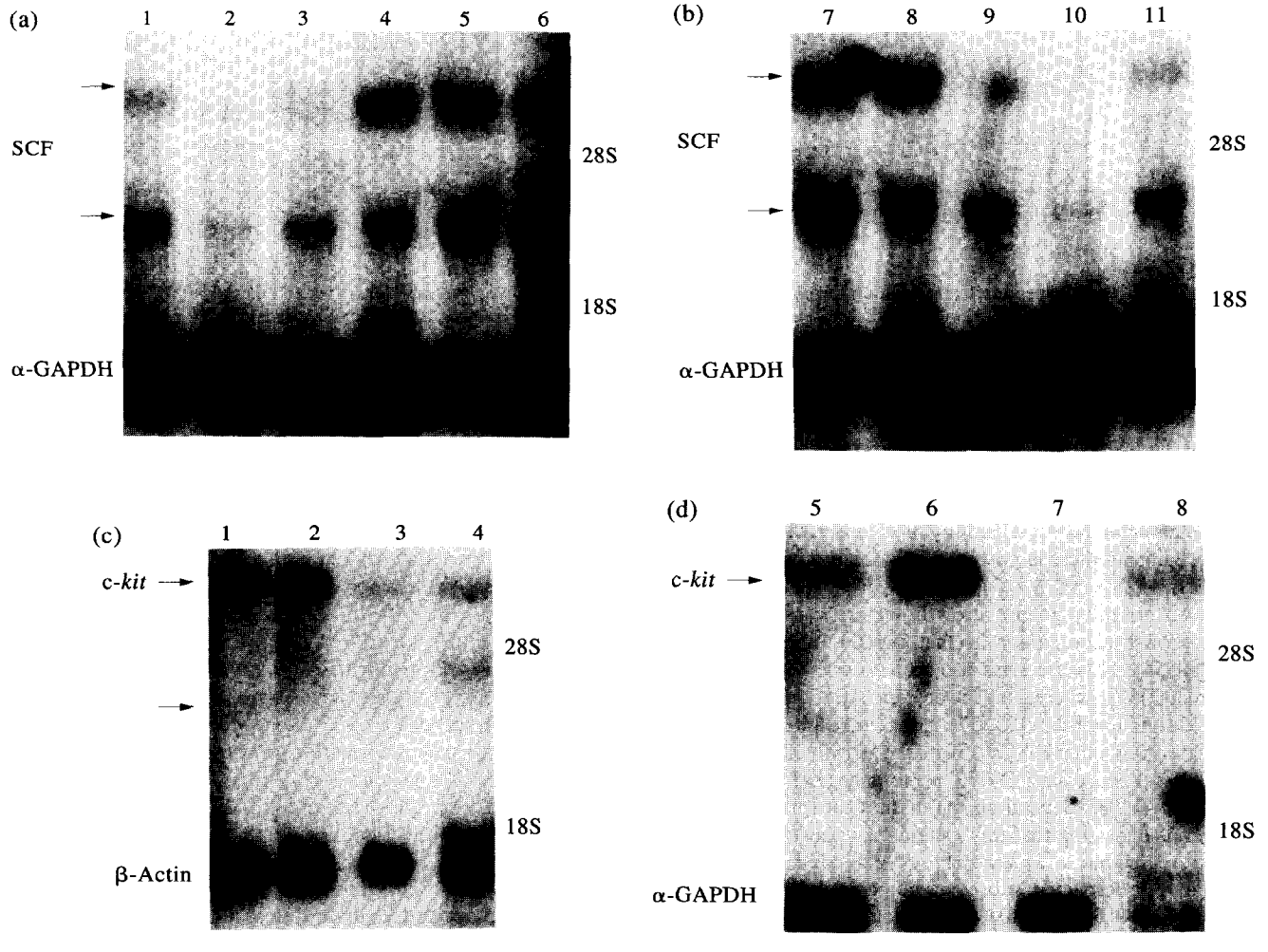

Figure 2. Detection of SCF and *C-KIT* transcripts by Northern blot analysis in lung cancer and melanoma cell lines. Expression of SCF (copies of either the membrane-bound, or the soluble form, or both) was detected in (a) all lung cancer (HTB 119, lane 1; HTB 171, lane 2; HTB 175, lane 3; HTB 53, lane 4; HTB 56, lane 5; HTB 120, lane 6) and (b) melanoma (SK-MEL-28, lane 7; A 375, lane 8; ST-MEL-11, lane 9; MeWo, lane 10; HAS lane 11) cell lines. Expression of *C-KIT* was observed also in (c) all SCLC (HTB 119, lane 1; HTB 120, lane 2; HTB 175, lane 3; HTB 171, lane 4) and (d) in three melanoma cell lines (ST-MEL-11, lane 5; MeWo, lane 6; SK-MEL-28, lane 8). HAS (lane 7) cell line was one of the *C-KIT* negative cell lines (d).

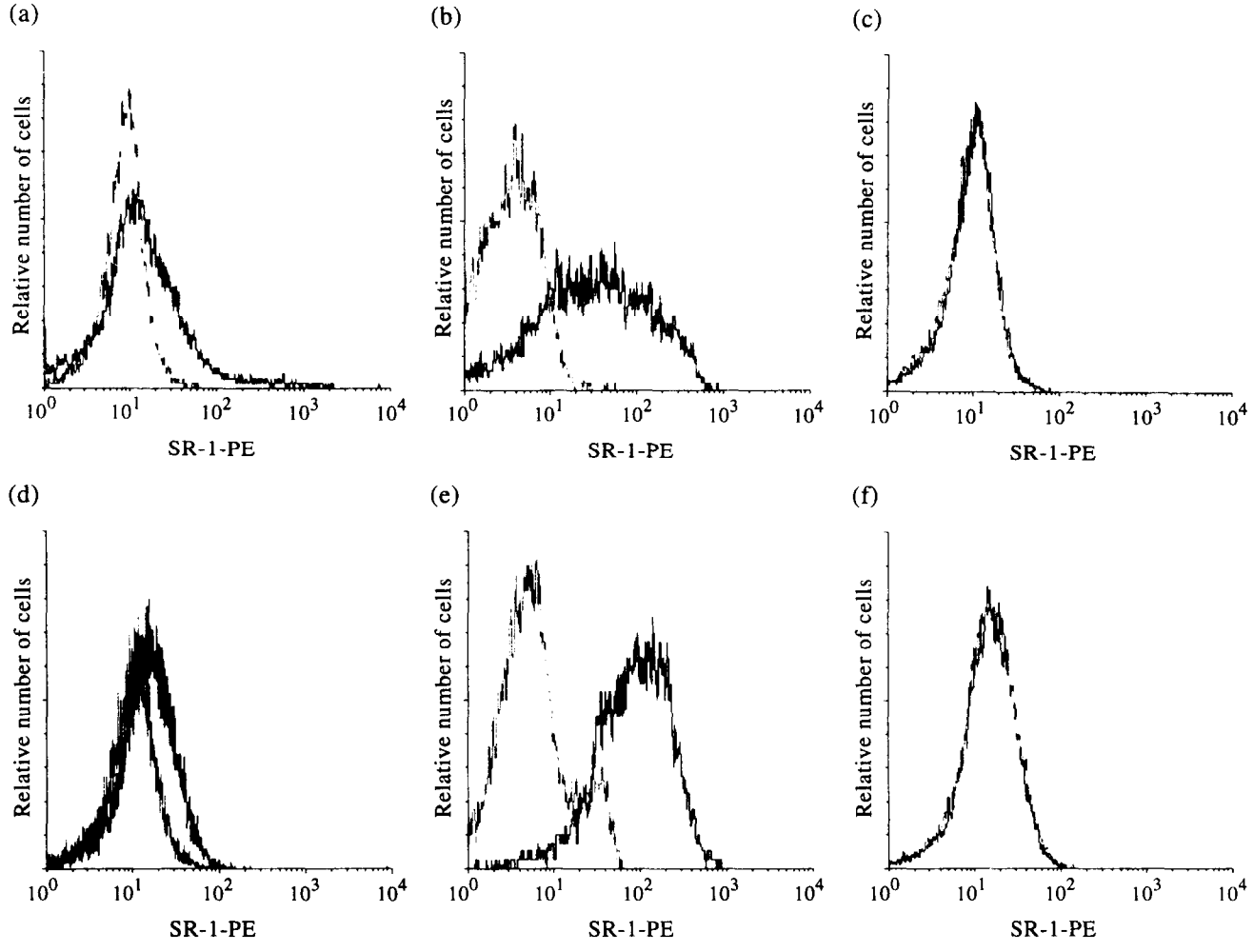

Figure 3. Kit-receptor protein expression on the cell line HEL (positive control; a), the human melanoma cell lines MeWo (b) and A375 (c), and the SCLC cell lines HTB 119 (d), HTB 120 (e), and HTB 53 (f). Cells were analysed by a FACScan analyser. For a detailed description see Materials and Methods.

between SCF and SCF-receptor expression in the cell lines studied, with SCF being more widely expressed than its receptor. Moreover, there was also transcript expression of both the ligand and the receptor in cell lines lacking expression at the protein level. This could reflect sensitivity problems with FACS analysis or mechanisms such as post-transcriptional downregulation of *C-KIT*.

Various haematopoietic growth factors can stimulate the clonal growth of some non-haematopoietic tumour cell lines *in vitro* [50–54]. This effect has also been observed in fresh tumour specimens [55], whereas at lower concentrations others could not find similar activity [56, 57]. Previous results by our laboratory have shown that rhSCF affects the growth of a glioblastoma (CRL 1620) expressing *C-KIT* proto-oncogene at mRNA and protein level [33]. Several normal tissues coexpress *C-KIT* and its ligand suggesting that this system may play a role in normal growth regulation in an autocrine or paracrine manner. However, a very restricted subset of human solid tumours, such as SCLC [34, 35, 45 ,58], gynaecological tumours [37] and melanomas [30], co-express this receptor–ligand system. Furthermore, Sekido and associates have reported a significant chemotactic response as well as moderate *in vitro* cell growth induced in SCLC cell lines (others than those tested in the present study) by the addition of rhSCF [59]. These data as well as our results suggest that SCF/*C-KIT* may play a biological role in the development of SCLC. The aberrant expression of *C-KIT* in SCLC might also enhance the migration of SCLC cells *in vivo*, resulting in the acquisition of a higher invasive and/or metastatic phenotype. The preferential expression of *C-KIT* in SCLC among various histological types of lung cancer could also explain why this tumour frequently metastasises to the bone marrow [58]. However, our study was not designed to estimate the frequency of SCF/*C-KIT* expression or SCF responsiveness in certain tumour histologies.

Previous experiments with HTB 119 (NCI-H 69) and HTB 120 (NCI-H 128) cell lines have shown the complete lack of a proliferative response to rhSCF, alone or in combination with G-CSF or IL-3, in colony formation assays. Turner and associates suggested that all available *C-KIT* receptors may be saturated by endogenously produced SCF [45]. This receptor saturation may explain why rhSCF does not stimulate clonal growth of the HTB 119 cell line. However, the substantial effect of rhSCF on the growth of HTB 120 cell line that we observed in both tritiated thymidine uptake and methylcellulose assays may reflect clonal evolution and presence of *C-KIT* products not saturated by autocrine SCF-binding. Alternatively, a mutation in the SCF protein which is known to be produced by this tumour cell line could result in ineffective binding of the endogenous cytokine to *C-KIT* receptor.

Although the loss of expression of *C-KIT* appears to be

associated with progression of some tumours, especially malignant melanomas [47, 60] and breast carcinomas [61], indicating that the Kit/ligand complex may participate as a negative control element during proliferation of the corresponding normal tissues, our study revealed *C-KIT* mRNA expression in three of the five melanoma cell lines tested. It should be mentioned here that *C-KIT* mutations can result in a ligand-independent activation of *C-KIT* receptor tyrosine kinase, thereby possibly contributing to excessive proliferation, aberrant differentiation and neoplastic transformation [62, 63]. SCF transcripts were also found in all melanomas with a weaker mRNA expression in MeWo. The latter was the only melanoma cell line which expressed *C-KIT* at the protein level, as detected by flow cytometric analysis, and could be reproducibly stimulated by rhSCF. However, the amount of the stimulatory effect was relatively minor. This cannot be explained by a lack of catalytic activity of the Kit kinase, as studies on autophosphorylation of Kit induced by exogenous ligand have shown. These experiments were performed by immunoprecipitation of KIT followed by phosphotyrosine blotting (H. Serve, unpublished data). The stimulatory effects of rhSCF on the clonal growth of the MeWo cell line were also inhibited dose-dependently by the tyrosine kinase inhibitor, genistein. In contrast, MeWo expresses low, but detectable levels of endogenous SCF, which might stimulate cell growth in an autocrine manner and thus diminish the effect of exogenous SCF. These results, together with the data reported by Mattei and associates [30], point to possible autocrine pathways in melanoma biology, a hypothesis which our study was not designed to test.

We have interpreted SCF as being a growth factor in a few cell lines. Since we have used the HTCA as our main assay system, it cannot be ruled out that the effects observed are due to a role of SCF as a survival factor. In addition, cell lines can acquire altered properties such as changes in their growth requirements and receptor expression. Thus, our results and some observations of other laboratories have to be interpreted with caution, and further studies using fresh tumour material are needed before final conclusions can be drawn.

In conclusion, SCF can be operative in growth or survival modulation of a few malignant non-haematopoietic cell lines, particularly SCLC and melanomas. Experiments *in vitro* and *in vivo* with a broader range of tumour histologies and fresh tumour material are needed to determine whether clinical application of SCF in conjunction with cytotoxic therapy represent potential hazards for cancer patients. Of particular interest are studies combining SCF with other haematopoietins, such as GM-CSF or G-CSF, which *in vivo* might also be used in combination with SCF. However, our extensive screening of SCF-tumour cell interactions makes potential hazards, such as tumour stimulation upon clinical use of rhSCF in conjunction with chemotherapy in cancer patients, unlikely for the majority of tumour histologies. Since both SCF and its receptor are expressed on some tumour cells, further studies should be performed in order to determine the possible role of an autocrine SCF/KIT loop in the immortalisation of various malignant cell lines, especially SCLC, and to give more insight into the growth regulating mechanisms of malignant tumours.

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