STEM CELL FACTOR AFFECTS TUMOUR PROGRESSION MARKERS IN METASTATIC MELANOMA CEELS

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ORIGINAL PAPER

Stem cell factor affects tumour progression markers in metastatic melanoma cells

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Abstract Stem cell factor (SCF), next to various relevant biological effects exerted on many cell types, is able to keep melanocyte homeostasis through its receptor c-kit. Only a minority of metastatic melanoma cells (MMC) express c-kit receptor, but c-kit positive MMC move more slowly towards tumour progression and have a more natural tendency to undergo apoptosis. In our study c-kit positive MMC from human melanoma metastases and a c-kit positive human melanoma cell line—SK-MEL-28—showed a clear-cut reduction of cytokines normally up-regulated along melanoma progression after SCF stimulation. SCF was also able to maintain all MMC and SK-MEL-28 cells in a well differentiated status with an increase in organellogenesis and in particular of melanosomes in various degree of differentiation, but it did not induce apoptosis as observed in other in vitro models. The increase of melanosomes matched an increase of tyrosinase production. SCF did not modify the expression of NOS while it enhanced the expression of HLA-DR molecules on MMC membranes. Taken altogether these data stress the biological activity of SCF as a cytokine which is able to maintain MMC in a well differentiated status, and suggest a more in depth evaluation of possible effects of SCF on melanoma cells.

Keywords c-kit receptor · Cytokines · Melanoma progression · Metastatic melanoma cells · Stem cell factor

Abbreviations

SCF	Stem cell factor
MMC	Metastatic melanoma cells
GM-CSF	Granulocyte-monocyte colony stimulating factor
TGF-β	Transforming growth factor-β
TNF-α	Tumour necrosis factor-α
IL-6	Interleukin-6
IL-7	Interleukin-7
IL-8	Interleukin-8
IL-10	Inteleukin-10
EM	Electron microscopy

Introduction

Stem cell factor (SCF), also known as mast cell growth factor or kit ligand is a glycoprotein growth factor [1–3] with important biological functions in both membrane-bound and soluble forms [2]. Its natural receptor is c-kit, a tyrosine kinase encoded by

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a protooncogene [4, 5]. Many different cell types are known to secrete SCF [6]: keratinocytes, fibroblasts, endothelial cells, bone marrow stromal cells. When either alone or combined with other cytokines such as GM-CSF [7], SCF is able to induce the maturation of primitive progenitors into mature, functional active cells and to enhance the transformations of monocytoid cells into dendritic cells. SCF cause melanoblasts to differentiate into melanocytes, and it is also highly mitogenic for melanocytes in vivo and in vitro [8]. The correct SCF/c-kit interaction is crucial for a regular differentiation of melanocytes during defined stages of embryogenic development and the homeostasis of mature melanocytes [9]. The SCF/c-kit signalling pathway plays a peculiar role in melanoma: melanocytes from metastatic melanoma often lose the expression of c-kit and there is an inverse correlation between c-kit expression and neoplastic progression [10, 11]. SCF/c-kit interaction is also a pathway through which melanoma cells undergo apoptosis thus reducing tumoural growth and metastatic invasion [12]. Only melanoma cells expressing c-kit undergo apoptosis in vivo and in vitro, so that the loss of c-kit seems to represent a crucial event in the progression of human melanoma [12].

In a previous study we demonstrated that some immunophenotypical markers, including c-kit, of cutaneous metastatic melanoma cells (MMC) could affect the chemosensitivity of these cells; therefore the behaviour of MMC appeared predictable according to their phenotype [13].

Melanoma cells constitutively produce a large amount of cytokines and growth factors, in which normal signalling pathways are often subverted [14]. Growth factors produced by tumoural cells act as autocrine molecules as they are able to bind to specific receptors expressed by the cells which can acquire growth autonomy in this way: the growth factors secreted by these cells can also influence neighbouring cells in a paracrine way [14–16]. Such growth factors and cytokines are variously expressed along melanoma progression and they play a role in the diverse array of phenotypic alterations observed in melanoma cells. Some cytokines, including SCF, can profoundly influence the secretion of some other cytokines and/or growth factors by melanoma cells [17].

We have investigated the effects of SCF on a human melanoma cell line (SK-MEL-28) and on 5 human MMC cultures, all characterized by c-kit expression [18], in order to establish their morphological and immunophenotypical profile, and the expression of some molecules and cytokines related to tumour progression [16, 19–22]. Our aim was to understand whether and to what extent SCF is able to affect melanoma progression markers.

Material and methods

Immunohistochemistry

Immunostaining was carried out on fresh specimens obtained from five melanoma metastases, using an alkakine phosphatase–anti-alkaline phosphatase (APAAP) method, with mouse monoclonal anti-human antibodies (MoAb) serving as primary reagents as listed in Table 1. Fragments were frozen at –80°C immediately following surgery; 6 μm cryostat serial sections were cut and immediately fixed with ice-cold acetone for 4 min, then subjected to staining. Thereafter sections were incubated with human AB-positive serum followed by primary MoAb for 2 h at room temperature. After washing for 10 min, sections were incubated for 40 min with rabbit anti-mouse immunoglobulin antiserum (RAM, 1:30), (Dako, Glostrup, Denmark), and then processed with alkaline phosphatase–anti-alkaline phosphatase complex (APAAP, 1:50) (Labometrics, Milan, Italy). In order to enhance the labelling intensity, RAM/APAAP incubation was repeated twice for surface antigens and 3 times for cytokines. Binding of the complex was revealed by hexazotized new fuchsin as chromogenic substrate (Merk, Darmstadt, Germany). Sections were then counterstained with Mayer's haematoxylin. Control sections were incubated with normal mouse IgG. Two investigators (SM and FP) read all tissue sections.

Table 1 Antibody panel

Antibody specificity	Working dilution	Source
c-kit[a]	1:30	Biosource
S-100 protein[bc]	1:100	Sigma
HMB-45[a]	1:45	Ortho Diagnostic System
HLA-Dr[ac]	1:10	Becton Dickinson
Ki-67[bc]	1:50	Dako
IL-6[a]	1:50	R & D Systems
IL-7[ac]	1:10	Genzyme Diagnostic
IL-8[a]	1:100	Pepro Tech EC LTD
IL-10[a]	1:30	Biosource International
TNF-alpha[a]	1:100	R & D Systems
TGF-beta[a]	1:100	R & D Systems
GM-CSF[a]	1:30	R & D Systems
i-NOS[ac]	1:500	Calbiochem
e-NOS[ac]	1:500	Calbiochem

[a] Mouse monoclonal antibody

[b] Rabbit polyclonal antibody

[c] Tested only on cell coltures in immunofluorescence

Discrepancies in the reading were resolved by a second parallel reading of the slides until consensus. The percentage of stained cells in each lesion was assessed according to five categories: 0–4%, 5–10%, 11–30%, 31–50%, and 51–100%. A cut-off of 5% positive cells was selected to identify positive lesions. At least five ×400 fields per section were examined. Staining for cytokines was considered as positive when melanoma cells showed cytoplasmic reactivity.

Cell cultures

Melanoma cells (MMC) were obtained from five subcutaneous metastases of five melanoma patients after obtaining informed consent and approval of the local ethical committee. Skin metastases were deprived of fat, washed many times in phosphate buffered saline and antibiotics and cut into many pieces. The first enzymatic digestion was performed with protease (Boeheringer/Mannheim, Mannheim, Germany) for 4 h at 37°C in RPMI 1640 medium with 1% penicillin/streptomycin (Sigma-Aldrich, Milan, Italy). The medium with the cells was then centrifuged and the supernatant was discarded. A second enzymatic digestion was then performed with collagenase (Sigma) for 3 h at 37°C in 5% CO_2 humidified atmosphere. The enzymatic action was stopped with a combination of Tris Base, NaCl and EDTA. The cells were then put in RPMI 1640 medium with 10% foetal calf serum (FCS) (Sigma), 1% penicillin/streptomycin (Sigma), 5% glutamine (Sigma), and 1% piruvate (Sigma) and cultured for numerous consecutive passages (at least 20 with an immunophenotypical control at each passage).

SK-MEL-28 cells were maintained in culture with the same medium as MMC

SCF (Pepro Tech) was added undiluted [7] at concentrations of 5 and 10 ng/ml to the culture medium for 72 h. MMC and SK-MEL-28 cells were evaluated before and after SCF treatment via immunofluorescence, electron microscopy, PCR and flow cytometry.

Immunofluorescence

MMC and SK-MEL-28 cells were grown on chamber slides (Nunc International, Roskilde, Denmark), rehydrated, blocked with 5% FCS, and incubated with primary MoAbs and polyclonal antibodies listed in Table 1. This incubation was protracted for 90 min at 37°C and was followed by either a fluorescein-isothiocyanate-conjugated goat anti-mouse (Sigma) or a fluorescein-isothiocyanate-conjugated goat anti-rabbit antibody (Sigma). The slides were then mounted with Gel/Mount (Biomeda, Augst, Switzerland) for 1 h at 37°C and observed under a Zeiss Axioskop microscope equipped for epifluorescence. Controls were incubated with normal mouse IgG or normal rabbit serum.

ELISA

MMC were cultured at 1×10^6 cells/well. Culture supernatants were collected before, 24, 48 and 72 h after SCF exposure. Cell culture supernatants were assayed for IL-10, IL-6, IL-8 and GM-CSF production with commercially available ELISA kits (R&D Systems, Minneapolis, MN), according to the manufacturer's guidelines. Plates were read in an ELISA reader (Biotech Instruments, Luton, UK), at a wavelength of 490 nm. Cytokine values were calculated from a standard curve of recombinant human IL-10, IL-6, IL-8 and GM-CSF. Detection limit was 1.5 pg/ml for IL-10, IL-6 and GM-CSF, and 3 pg/ml for IL-8. Each experiment was performed in double.

Electron microscopy

MMC and SK-MEL-28 cells were fixed in a mixture of 2% formaldehyde and 2.5% glutaraldehyde in 0.1 mmol/l cacodylate buffer, pH 7.4, at 5°C for 3 h, followed by postfixation in 1% OsO_4 in phosphate buffer, pH 7.4 at room temperature for 2 h. The specimens were dehydrated in a graded acetone series, passed through propylene-oxide and embedded in epon 812. A sequential number of semithin sections were coloured with toluidine blue, while ultrathin sections were stained with uranyl acetate followed by bismuth subnitrate or lead citrate, and examined in a Jeol 1010 (Tokyo, Japan) at 80 kV.

PCR

Treated and control melanoma cells were subjected to RNA extraction with the RNeasy Mini Kit (Qiagen) and RNA samples were stored at –20°C until dosage (within one week). The concentration and purity of RNA were detected spectrophotometrically after Dnase treatment. Each sample was run in triplicate and each experiment included a positive and a negative control (with no template).

Each sample (400 ng RNA) was reverse-transcribed using TaqMan RT-PCR kit (PE Applied Biosystems),

in a final volume of 80 µl containing 1× TaqMan RT buffer (500 mM KCl, 0.1 mM EDTA, 100 mM Tris–HCl, pH 8.3, 600 mM of passive reference ROX), 5.5 mM $MgCl_2$, 500 µM of each dNTP, 2.5 µM of random hexamer, 0.4 U/µl of Rnase Inhibitor, 1.25 U/µl of Multiscribe Reverse Transcriptase.

The following primers and probe were selected by the ''Primer Express'' software (PE Applied Biosystems) for tyrosinase mRNA measurement with quantitative real time PCR: forward primer 1092 5′-TTCAG GTTTAGAAATACACTGGAAGG-3′, reverse primer 1172 5′-AATGTGCATGCTGCTTTGAGA-3′, and the FAM labelled specific fluorescent probe 1119 5′-TTTGCTAGTCCACTTACTGGGATAGCGGA TG-3′. Twenty five nanogram of cDNA (5 µl/tube) were used for each sample and 20 µl/tube of PCR mix containing 1× TaqMan Universal Master Mix, 300 nM of each primer and 200 nM tyrosinase fluorescent probe were added. The thermal cycling conditions were 2 min at 50°C, 10 min at 95°C, and then 40 cycles at 95°C for 15 s, 60°C for 1 min. PCR amplification was performed with the ABI Prism 7700 Sequence Detector (PE Applied Biosystems). To calculate the expression of tyrosinase mRNA in each sample we referred to an external reference curve generated with total RNA extracted from the human SK-MEL-28 melanoma cell line. The reference curve ranged from 25 ng to 0.025 pg of reverse-transcribed total SK-MEL-28 RNA. Tyrosinase mRNA concentration was expressed as pg SK-MEL-28 RNA equivalents/µg total RNA.

Flow cytometry

MMC were cultured with increasing concentrations of SCF (0, 5, 10 ng/ml) for 72 h. Then cells at a concentration of 1 million/ml were labelled with mouse anti-human HLA-DR-FTIC (L243, IgG2a) from Becton Dickinson (San Josè, CA). MoAb dilutions and washing steps were performed in phosphate buffered saline (EuroClone) containing 2% FCS and 2 mM EDTA. Isotype-matched Ab was used as negative control. Melanoma cells were acquired using FACSort and analysed using the CellQuest software (Becton Dickinson Immunocytometry Systems, San Jose, CA). Seven-amino-actinomycin D (7-AAD; VIA-PROBE™, BD Pharminger San Diego, CA) was used to exclude dead cells from analysis.

Statistical analysis was performed using the SPSS Inc. Software (version 10.1). The comparison between treated and control cells was performed using Wilcoxon's matched pairs signed rank test. A level of $P < 0.05$ was considered significant.

Results

Immunophenotypic and phenotypic markers

All five cutaneous metastases expressed c-kit protein at a percentage of 31–50% of neoplastic cells (Fig. 1) on tissue sections; accordingly, in basal conditions the large majority (>80%) of MMC were c-kit positive (Fig. 2), and SK-MEL-28 cells exhibited a similar reactivity. We therefore assumed that our melanoma metastases were strongly c-kit positive, which is certainly an infrequent finding in MMC [10, 23]. Expression of HMB-45 antigen was detected on metastatic tissue (lining 31–50% of melanoma cells in two cases and 11–30% in the others), and on MMC and SK-MEL-28 cells in basal condition (Table 2). MMC and SK-MEL-28 cells also stained for S-100 protein (Fig. 3) and HLA-DR antigens, whose positivity remained after SCF treatment (Table 2). Basal Ki-67 staining of cultured cells dropped from 20–30% to less than 10% reactivity upon SCF treatment (Table 2).

Flow cytometry confirmed HLA-DR expression of melanoma cells suggesting a mild dose-dependent, although not statistically significant, up-regulation of HLA-DR molecules after increasing SCF stimulation (Fig. 4).

Tyrosinase mRNA (Fig. 5) was significantly higher in cells treated with 5 ng SCF (median 1840 pg SK-MEL-28 RNA/µg total RNA) in comparison to the untreated control cells (median 1480 pg SK-MEL-28 RNA/µg total RNA) ($P = 0.04$). 10 ng SCF also produced an additional increase in tyrosinase mRNA production (median 2480 pg SK-MEL-28 RNA/µg total RNA), which also appeared significantly higher compared to the untreated cells ($P = 0.03$).

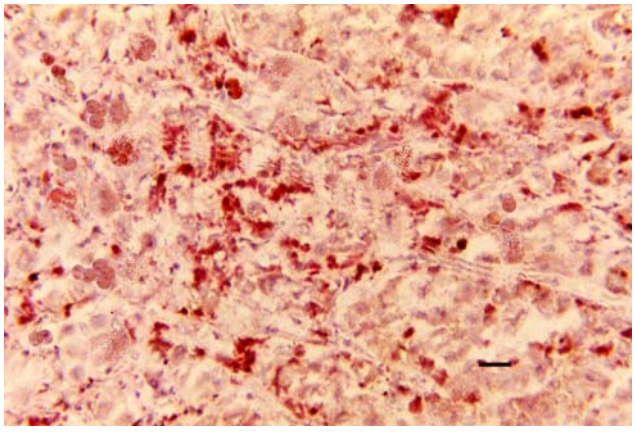

Fig. 1 Melanoma metastasis: c-kit positive clusters of neoplastic cells (APAAP; 6 mm bar = 30 µm)

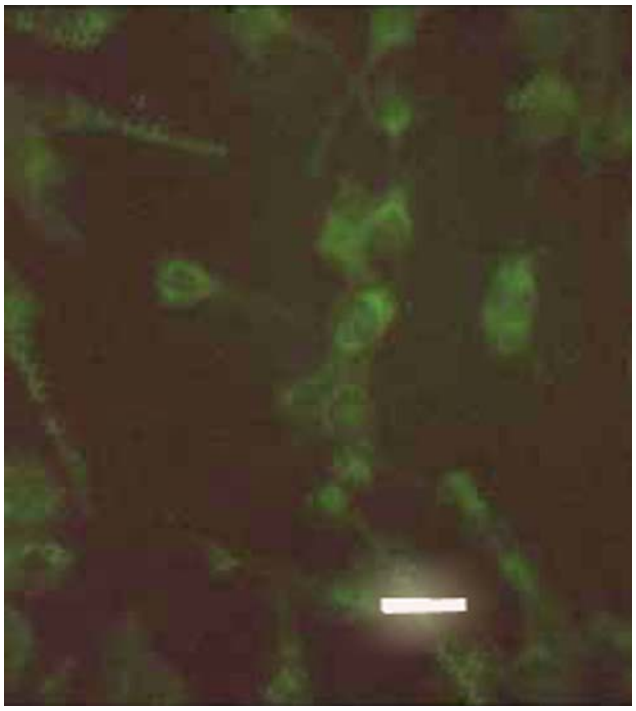

Fig. 2 Metastatic melanoma cells (MMC) staining for c-kit receptor (immunofluorescence; 12 mm bar = 60 μm)

Cytokines

All five metastases exhibited some reactivity for IL-6, IL-8, IL-10, GM-CSF and TGF- beta on tissue sections with immunohistochemistry. TGF-beta stained a majority of cells (>51%) (Fig. 6) in one case and 11–30% of neoplastic cells in four cases; IL-6 (Fig. 7) and GM-CSF were found in 11–30% of melanoma cells in one case and in 5–10% in the others. IL-10 and IL-8 (Fig. 8) were expressed by all lesions at a percentage of 5–10%, and TNF-alpha was detected only in one case (5–10% of positive cells).

Table 2 Results of immunofluorescence staining

	SK-MEL 28	MMC	SK-MEL 28 + SCF	MMC + SCF
S-100	+	+	+	+
HMB-45	+	+	+	+
HLA-Dr	+	+	+	+
Ki-67	+	+	±	±
IL-6	+	+	+	+
IL-7	+	+	+	±
IL-8	–	+	–	±
IL-10	–	+	–	–
GM-CSF	+	+	–	–
TNF-alpha	+	–	–	–
TGF-beta	+	+	±	–
i-NOS	+	+	+	+
e-NOS	+	+	+	+

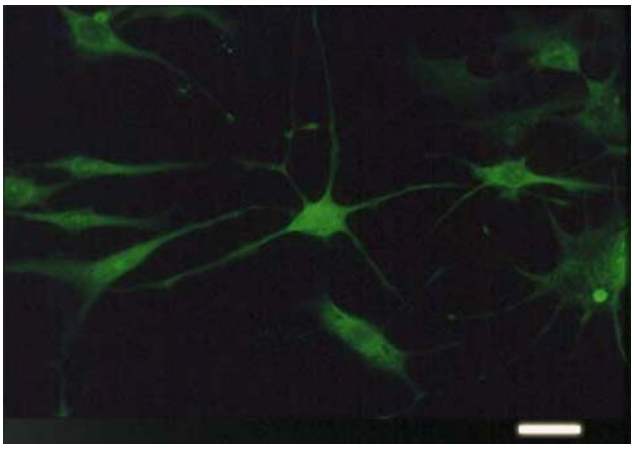

Fig. 3 MMC staining for S-100 protein (immunofluorescence; 6 mm bar = 30 μm)

These results were well confirmed with immunofluorescence on cultured cells (Table 2).

In basal conditions most of the cells were positive for IL-6, IL-7, TGF-b (Fig. 9) and some cells for GM-CSF, while IL-8 and IL-10 (Fig. 10) were only found in MMC (and not in SK-MEL-28 cells); TNF-alpha was expressed by SK-MEL-28 cell line and not by MMC. After SCF treatment, TGF-beta was undetectable both in MMC and SK-MEL-28 cells, IL-7 decreased in MMC but was still present in SK-MEL-28 line; TNF-alpha cleared in SK-MEL-28 cells; IL-8 was reduced still, while IL-10 and GM-CSF disappeared in MMC.

Both SK-MEL-28 cells and MMC expressed the two isoforms of nitric oxide synthase, cNOS and iNOS at basal level, and this staining was maintained after SCF treatment.

Cytokine production by three MMC is shown in Fig. 11. High basal levels of IL-8 decreased sharply under SCF stimulus at 72 h ($P < 0.03$). Basal IL-10 levels decreased ($P < 0.05$ at 72 h) while IL-6 values

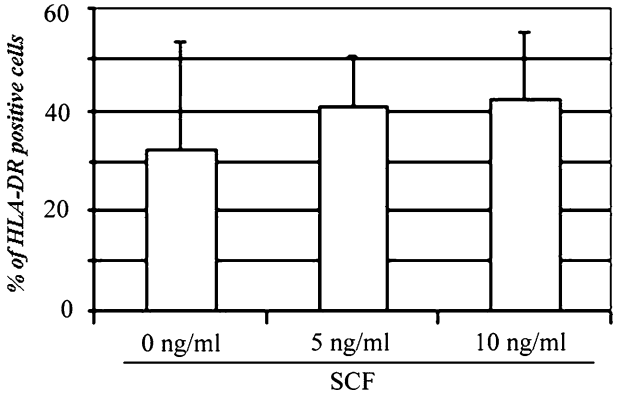

Fig. 4 HLA-DR molecules appear more expressed on MMC in presence of SCF

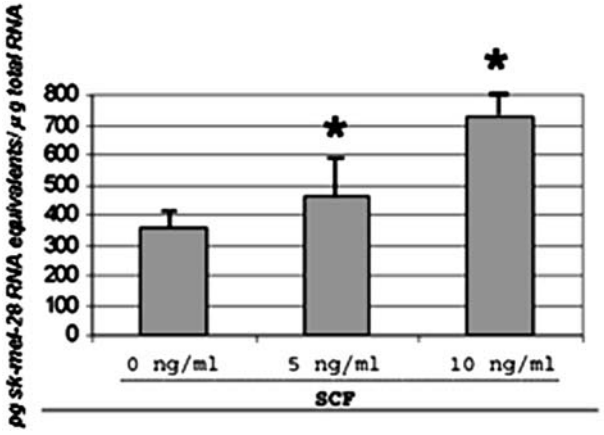

Fig. 5 Tyrosinase expression in MMC increases significantly according to SCF dosage (the asterisk represents a significant increase)

showed only a slight reduction upon SCF treatment, and low basal values of GM-CSF became nearly undetectable at all times upon SCF treatment ($P < 0.05$). These results appear to agree with the results observed in immunofluorescence before and after SCF.

Ultrastructure

In basal conditions MMC and SK-MEL-28 were characterized by a homogeneous morphology: they were dendritic, with an indented nucleus (Fig. 12). Their cytoplasm appeared rich in organelles, as smooth and rough endoplasmic reticulum, Golgi apparatus and many mitochondria. Some melanosomes at various degree of differentiation were also detectable in the majority of cells (Fig. 13). Upon SCF treatment the cells showed a smooth profile with a reduction of dendricity (Fig. 14), and an increase of organellogenesis—especially in MMC; an increased number of melanosomes (compared to control) was observed at various degrees of differentiation close to the Golgian and perigolgian area of the cytoplasm (Fig. 15). These modifications were detected especially upon higher dosage of SCF. There were nor membranous or organelle alterations typical of apoptosis.

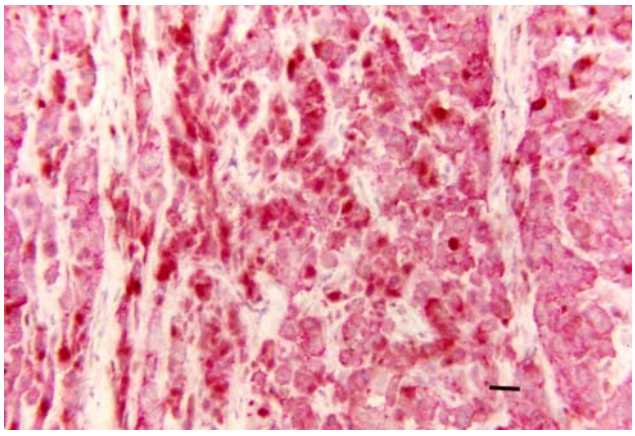

Fig. 7 Melanoma metastasis: neoplastic cells in the section express IL-6 (APAAP; 6 mm bar = 30 µm)

Discussion

In this study the effects of SCF on MMC and SK-MEL-28 melanoma cell line (all expressing c-kit receptor) were investigated. Both SK-MEL-28 cells and MMC are able to maintain a well differentiated status upon treatment with SCF as documented by immnunophenotypical markers, such as HMB45, S100 and HLA-DR antigens. A reduced HLA-DR expression on melanoma cells after various cytokine stimuli (but not SCF) has been reported in literature as an important

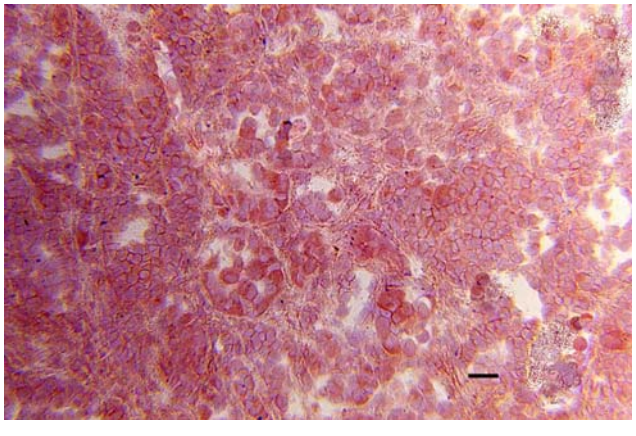

Fig. 6 Melanoma metastasis: most melanoma cells stain for TGF-β (APAAP; 6 mm bar = 30 µm)

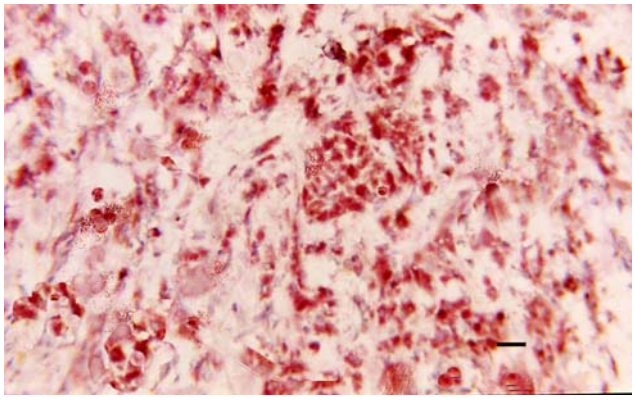

Fig. 8 Melanoma metastasis: neoplastic cells stain for IL-8 (APAAP; 6 mm bar = 30 µm)

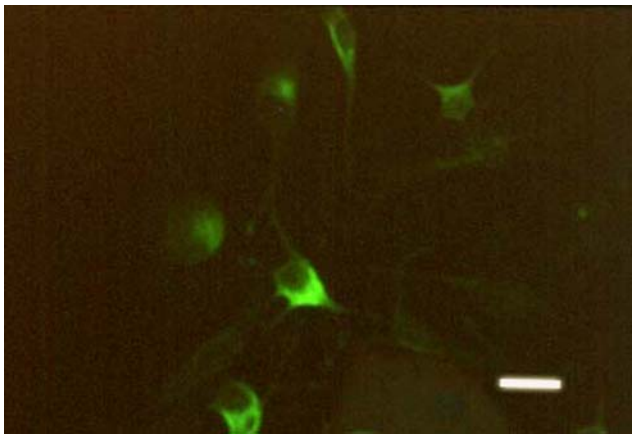

Fig. 9 MMC: the cytoplasm of the cells shows a clear-cut positivity for TGF-β (immunofluorescence; 12 mm bar = 60 μm)

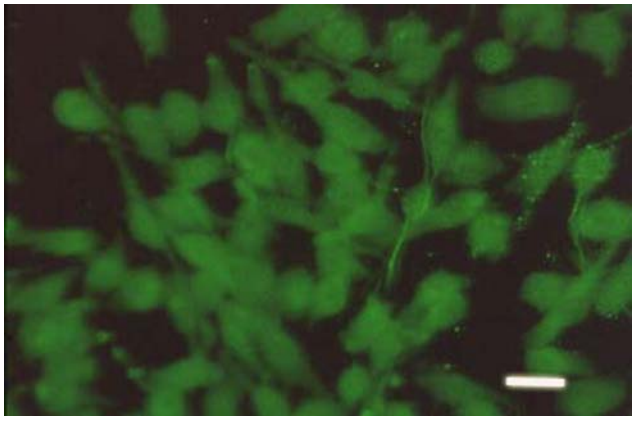

Fig. 10 MMC: many cells with positive staining for IL-10 (immunofluorescence; 12 mm bar = 60 μm)

feature associated with the development of metastatic phenotype [24]. In our study SCF increased HLA-DR antigen expression on MMC, demonstrating an opposite effect on second class MHC, compared to inflammatory cytokines [24].

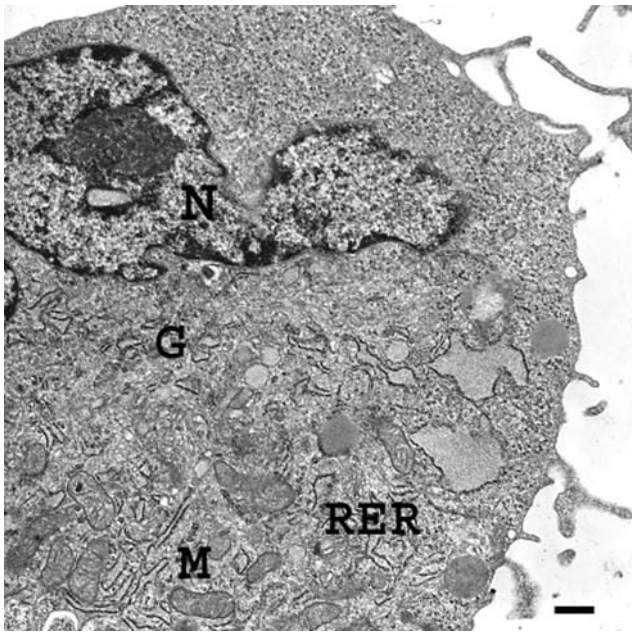

Fig. 12 MMC: the cell has the typical shape of a melanocyte: an indented nucleus with many organelles within the cytoplasm and many dendrites along the cytoplasmic membrane [electron microscopy (EM); 5 mm bar = 1 μm)]. N = nucleus; RER = rough endoplasmic reticulum; G = Golgi apparatus; M = mitochondria

Moreover, SCF-conditioned MMC under electron microscopy showed the presence of many organelles within the cytoplasm, and particularly a high number of melanosomes, suggesting a trend toward a more differentiated and higher melanogenetic status. Both SK-MEL-28 cells and MMC increased tyrosinase expression after SCF, compared to untreated cells. This is in agreement with the higher number of melanosomes within the MMC-treated cells in which melanosomes in various stages of maturation were found. It can be supposed that SCF is capable of inducing synthesis of tyrosinase in our melanoma cells. It is noteworthy that SCF is able to induce the transformation of monocytoid

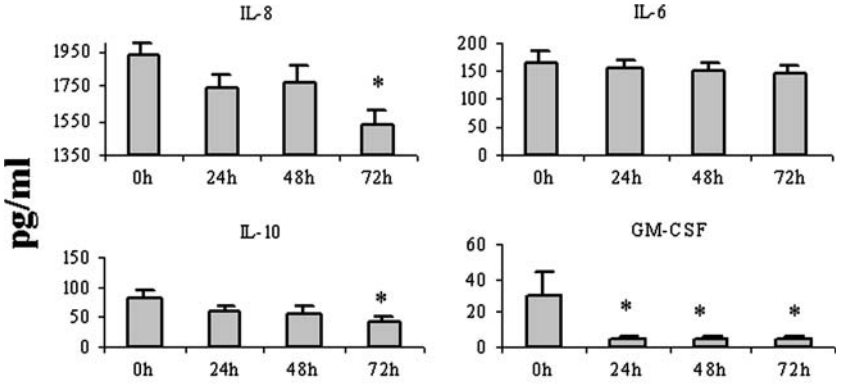

Fig. 11 Cytokine evaluation on cell surnatants expressed as pg/ml (evaluation performed on 3 MMC, each bar representing the average ± SD of 3 MMC) before and after SCF treatment (10 ng/ml; evaluation performed at 0, 24, 48 and 72 h). IL-8, IL-10 and GM-CSF secretion decreases after SCF treatment (the asterisk represents a significant reduction)

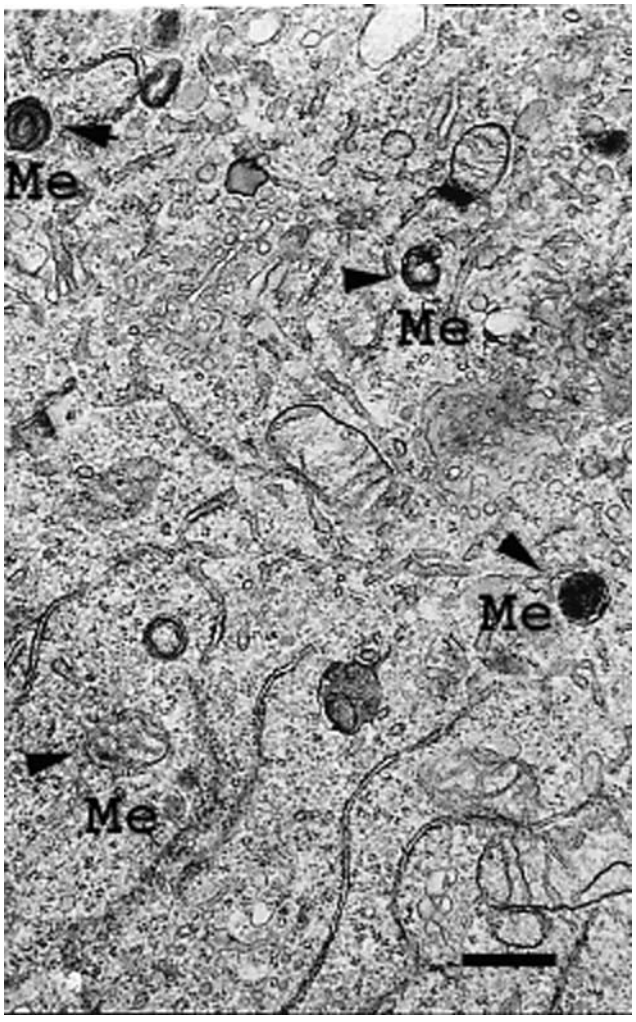

Fig. 13 MMC: Few melanosomes within the cytoplasm at various degree of differentiation before SCF treatment (EM; 16 mm bar = 0.5 μm). Me = melanosomes; arrowheads pointing melanosomes

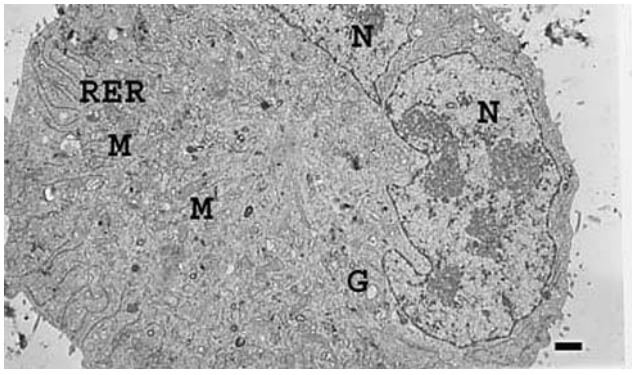

Fig. 14 MMC: smooth and rough endoplasmic reticulum, Golgi apparatus and many mitochondria within the cytoplasm upon treatment with SCF (EM; 5 mm bar = 1 μm). N = nucleus; RER = rough endoplasmic reticulum; G = Golgi apparatus; M = mitochondria

cells into dendritic cells, enhancing dendricity [7], while seems to induce a smooth cell profile in MMC.

SCF-treated MMC also show a reduced expression of some cytokines associated with melanoma progression, such as IL-7, IL-10, IL-8, GM-CSF and TGF-beta [16, 19, 20], suggesting that SCF is able to induce a less malignant phenotype in conditioned cells; this result is in agreement with the observed decrease in proliferation rate, assessed by Ki67 antigen. TNF-alpha, which is not considered a progression marker of melanoma [16], appeared to be reduced as well. A similar cytokine decrease was observed in SCF-treated SK-MEL-28 cells, except the expression of IL-7 and TGF-beta which were maintained or slightly reduced. Surnatant evaluation of cytokines secreted by MMC paralleled the results observed in immunofluorescence, and appeared more evident for IL-8, which is considered an autocrine growth factor for in vitro melanoma cells [25].

MMC expressed both cNOS and iNOS in basal conditions and after treatment with SCF. While cNOS needs the presence of Ca2+ to be activated, iNOS is Ca2+ independent and can be activated by many cytokines [26]. NO role in neoplastic and apoptotic processes is still controversial [26]. In fact, iNOS expression was found either to correlate with malignancy [22] or not to correlate clearly in human melanocytic lesions in vivo [27], while an inverse correlation with tumour progression has been documented in vitro [28]. Furthermore, a different response of iNOS to cytokine (as TNF-alpha or IFN-gamma) induction has been reported by normal melanocytes and melanoma cells [29]. NO role does not seem

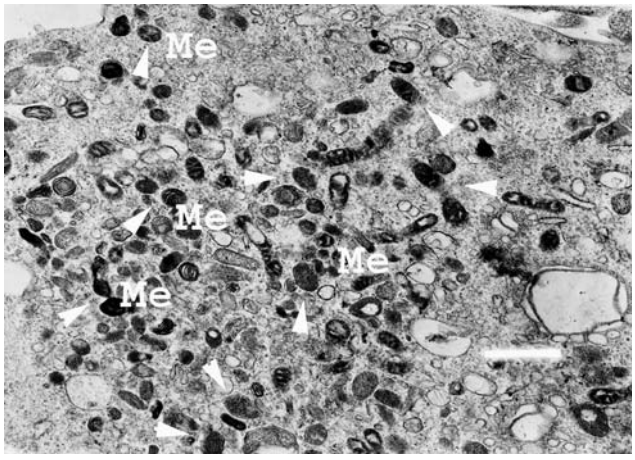

Fig. 15 MMC: a rich melanogenesis with melanosomes at various degree of differentiation upon treatment with SCF (EM; 16 mm bar = 1 μm). Me = melanosomes; arrowheads pointing melanosomes

clearly assessed in melanoma progression so far and in our study it appears not to be affected by SCF treatment. As the expression of both cNOS and iNOS was present in basal conditions in all 5 MMC, it could be hypothesized that their expression is strictly related to their genotype and not induced by any cytokine addition as stressed by Fecker et al. [29].

Since NO is reported to exert anti-apoptotic activity in melanoma cells [30], its expression in our cells appears to agree with the absence of apoptotic changes observed in SCF-treated MMC under electron microscopy. In fact, SCF has been reported to induce apoptosis in enforced c-kit expressing MMC [12], but apoptosis was not detected in our c-kit positive MMC after SCF. It is also possible that apoptosis has not been detected in our MMC because they expressed c-kit receptor in vivo constitutively, whereas MMC lines studied by Huang et al. [12] had been transfected in vitro with c-kit gene, thus creating a peculiar biological behaviour.

The relevant result of this work is that SCF seems capable of maintaining MMC in a well differentiated status, as stressed by morphology and immunophenotype, and of reducing or abrogating the production of cytokines strictly related to melanoma progression. This SCF activity could give rise to in depth expansion of these preliminary observations, in order to evaluate possible further effects on the activity of MMC.

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