available at www.sciencedirect.com

ScienceDirect

journal homepage: www.ejconline.com

Functional activity of CXCL8 receptors, CXCR1 and CXCR2, on human malignant melanoma progression ☆

Chiara Gabellini [a], *Daniela Trisciuoglio* [a], *Marianna Desideri* [a], *Antonio Candiloro* [a], *Ylenia Ragazzoni* [a], *Augusto Orlandi* [b], *Gabriella Zupi* [a], *Donatella Del Bufalo* [a,*]

[a] *Experimental Chemotherapy Laboratory, Regina Elena Cancer Institute, Via delle Messi d'Oro 156, 00158 Rome, Italy*
[b] *Anatomic Pathology, Tor Vergata University, Rome, Italy*

ARTICLE INFO

Article history:
Received 2 April 2009
Received in revised form 14 July 2009
Accepted 17 July 2009
Available online 13 August 2009

Keywords:
Melanoma
Pathologic neovascularisation
Chemotaxis
Cell proliferation
Neoplasm invasiveness
Interleukin-8
CXCR1
CXCR2
Cell hypoxia

ABSTRACT

We examined the autocrine/paracrine role of interleukin-8 (CXCL8) and the functional significance of CXCL8 receptors, CXCR1 and CXCR2, in human malignant melanoma proliferation, migration, invasion and angiogenesis. We found that a panel of seven cell lines, even though at different extent, secreted CXCL8 protein, and expressed CXCR1 and CXCR2 independently from the CXCL8 expression, but depending on the oxygen level. In fact, hypoxic exposure increases the expression of CXCR1 and CXCR2. The cell proliferation of both M20 and A375SM lines, expressing similar levels of both CXCR1 and CXCR2 but secreting low and high amounts of CXCL8, respectively, was significantly enhanced by CXCL8 exposure and reduced by CXCL8, CXCR1 and CXCR2 neutralising antibodies, indicating the autocrine/paracrine role of CXCL8 in melanoma cell proliferation. Moreover, an increased invasion and migration in response to CXCL8 was observed in several cell lines, and a further enhancement evidenced under hypoxic conditions. A CXCL8-dependent *in vivo* vessel formation, evaluated through a matrigel assay, was also demonstrated. Furthermore, when neutralising antibodies against CXCR1 or CXCR2 were used, only the involvement of CXCR2, but not CXCR1 was observed on cell migration and invasion, while both receptors played a role in angiogenesis.

In summary, our data demonstrate that CXCL8 induces cell proliferation and angiogenesis through both receptors and that CXCR2 plays an important role in regulating the CXCL8-mediated invasive and migratory behaviour of human melanoma cells. Thus, blocking the CXCL8 signalling axis promises an improvement for the therapy of cancer and, in particular, of metastatic melanoma.

1. Introduction

The molecular events that explain malignant melanoma progression have only been partially characterised. The progression of melanoma is accompanied by the deregulated expression of a number of proteins, including the chemokine interleukin-8 (CXCL8/IL8) and its receptors.[1] CXCL8 is secreted by a variety of human normal and tumoural cells, and in particular this multi-functional cytokine is involved in many important biological features, such as the formation, development and response to chemotherapy of melanoma.[2,3] Radial to vertical growth phase transition of melanoma and worse

☆ Grant support: Supported by grants from the Italian Association for Cancer Research (D.D.B. and A.O.), the Italian Ministry of Health (G.Z., D.D.B.) and FIRB-MIUR (G.Z.). C.G. is a recipient of a fellowship from the Italian Foundation for Cancer Research.

* *Corresponding author*: Tel.: +39 06 52662575/37; fax: +39 06 52662592.
E-mail addresses: delbufalo@ifo.it, ddelbufalo@gmail.com (D. Del Bufalo).

0959-8049/$ - see front matter
doi:10.1016/j.ejca.2009.07.007

prognosis are associated with CXCL8 overexpression,[4,5] increased CXCL8 serum concentration in melanoma patients is associated with tumour progression and survival,[6] and a positive correlation between CXCL8 expression and Clark level was observed in primary cutaneous melanoma.[7] We also recently demonstrated the modulation of CXCL8-dependent angiogenesis by bcl-xL in tumour cells, including melanoma.[8,9] The role of CXCL8 in tumour progression has been attributed to its ability to act as an autocrine/paracrine cytokine that enhances melanoma cell proliferation,[1] chemotaxis,[10] extravasation[11] and angiogenesis.[12] Studies with CXCL8 neutralising antibodies support the concept that the complex role of CXCL8 and its receptors in *in vivo* growth and development of melanoma goes far beyond the autocrine growth stimulation mechanism, but clearly involves the induction of neoangiogenesis and metastasis.[13]

The biological effects of CXCL8 are mediated through the binding of CXCL8 to two cell surface receptors CXC chemokine receptor 1 (CXCR1, CXCL8R1 or type A) and CXC chemokine receptor 2 (CXCR2, CXCL8R2 or type B), which besides their considerable structural similarity, show distinct ligand-binding pharmacology.[14] While CXCR1 is more specific, binding only CXCL8 and granulocyte chemotactic protein-2, the promiscuous CXCR2 binds multiple CXC chemokines.[14] These receptors are widely expressed on normal cells, such as leucocytes, keratinocytes and endothelial cells[15,16], as well as various tumour cells, including melanoma.[17,18] Despite many overlapping functions, CXCR1 and CXCR2 activate different signalling pathways, thus exhibiting different physiological roles strictly dependent on the cell type and tumour histotype.[12,17,19–21] Given that several but controversial reports demonstrated CXCR1 and CXCR2 involvement in different CXCL8-mediated biological functions of melanoma,[17,18] in this paper we examined the autocrine/paracrine role of CXCL8 and the functional significance of CXCL8 receptors, CXCR1 and CXCR2, in human malignant melanoma proliferation, migration, invasion and angiogenesis.

2. Materials and methods

2.1. Cell lines and treatments

The melanoma cell lines JR8, M14, PLF2, M20 and SAN were established from human metastatic lesions. A375SM and SBCL7 were derived by *in vivo* selection in nude mice of two different cell lines (A375 and SBCL1, respectively) established from human metastatic lesions.

Recombinant human CXCL8 (rhCXCL8, R&D Systems, Minneapolis, MN) was freshly prepared for each experiment, following the manufacturer's instructions. Exposure to hypoxia (1% oxygen concentration, 24 h) was performed as previously reported.[22]

2.2. Analyses of protein expression

Evaluation of CXCL8 protein in the serum-free conditioned medium (CM) was done by ELISA (R&D Systems). Immunoblotting of cellular lysates or CM proteins was performed as previously reported.[8,22]

2.3. Cytofluorimetric analysis

Cytofluorimetric analysis was performed as previously reported,[23] using fluorescein-conjugated (FITC) anti-CXCR1 or anti-CXCR2 antibodies (1:2.5, R&D Systems). Neutrophils were used as positive control. For all the samples, 20,000 events of a propidium iodide (Sigma, St. Louis, MI, USA)-negative cell population were acquired (FACScan, Becton Dickinson, Franklin Lakes, NJ, USA). Background green fluorescence in the cells was determined by incubation with FITC-IgG2a isotype control.

2.4. Proliferation, chemoinvasion and chemotaxis assays

Cell proliferation was evaluated by a colorimetric assay.[24] Chemoinvasion and chemotaxis assays were performed using 10 ng/ml rhCXCL8 as chemoattractant in Boyden chambers as previously described.[24,25] PBS as chemoattractant was used as negative control. The results were expressed as OD at 540 nm. CXCL8 protein in the CM of cells was neutralised using anti-CXCL8 antibody (0.2 μg/ml, R&D Systems), while CXCR1 and CXCR2 functions were inhibited using antibody neutralising CXCR1 or CXCR2, respectively (1 μg/ml Invitrogen, Carlsbad, CA, USA). Normal rabbit IgG (Pierce Biotechnology Inc., Rockford, IL, USA) was used as control.

2.5. In vivo matrigel assay

In vivo matrigel assay and quantification of the haemoglobin (Hb) content, expressed as OD/g matrigel plug, were performed as previously reported[8], using cell suspensions in the presence of 5 μg/ml antibodies neutralising CXCL8 (R&D Systems), CXCR1 or CXCR2 (Invitrogen). Matrigel plugs containing PBS were used as negative control. All procedures involving animals and their care were conducted in conformity with the institutional guidelines, which are in compliance with national and international laws.[25] For quantification of microvessel density (MVD), matrigel plugs were placed in 4% buffered formalin for 24 h, processed and embedded in paraffin. Serial 4 μm-thick sections were stained with Haematoxylin-Eosin, Masson's Trichrome or employed for immunohistochemistry. The MVD was calculated by counting the mean number of microvessel/field at 200× magnification (HPF) in at least six fields for each plug by two pathologists in a blinded manner; the interobserver variability was <5%. The representative images are presented at 200× HPF. To confirm the endothelial phenotype, an immunostaining[26] with a rabbit polyclonal anti-Von Willebrand's factor antibody (1:200, DAKO, Denmark), which also crossreacts with murine Von Willebrand's factor[27] was performed for 30 min at room temperature, with positive and negative controls. The representative images are presented at 400× HPF for control muscle biopsy and matrigel plugs section containing cells, and 200× HPF for matrigel plugs which do not contain cells.

2.6. Statistical analysis

The results were expressed as the mean ± standard deviation (SD) from *n* determinations. The significance of differences

between the means was determined with the Student's two-tailed t test (PSP6 software). Values of $p < 0.05$ were accepted as statistically significant and are expressed as symbols (* or §): 1 symbol $p < 0.05$; 2 symbols $p < 0.01$; 3 symbols $p < 0.001$. All the experiments were repeated at least three times and each point was tested in sextuplicate (cell proliferation and Matrigel assays) or in triplicate (migration and invasion).

3. Results

3.1. *CXCL8 protein secretion and cell surface expression of CXCR1 and CXCR2*

We first analysed the secretion of CXCL8 protein and the expression of CXCR1 and CXCR2 on a panel of seven human melanoma cell lines. As reported in Fig. 1A, CXCL8 protein was differently secreted in the various lines: a very low level was detected in cell-free CM of M20 cells (40 pg/10^6 cells/24 h), while A375SM and SAN cells produced the highest levels (2490 and 2972 pg/10^6 cells/24 h, respectively) and PLF2, M14, JR8 and SBCL7 showed intermediate levels (from 190 to 1242 pg/10^6 cells/24 h). Western blot analysis of CXCL8 expression in the CM of A375SM, SBCL7 and M20 confirmed the results obtained from ELISA (Fig. 1B). As evidenced by cytofluorimetric analysis performed two days after seeding, all the cell lines expressed both CXCR1 and CXCR2 on the cell surface, the percentage of positive cells ranging from about 50% to 90% and from 60% to 90% for CXCR1 and CXCR2, respectively (Fig. 1C). To evaluate whether the expression of both receptors was related to the proliferative status of the cells and oxygen availability, the expression of CXCR1 and CXCR2 was evaluated at different days after seeding or under hypoxic condition. As reported in Fig. 1D, a time-dependent increase in the expression of both receptors was observed in JR8 cells passing from days 1 to 4: the percentage of positive cells ranged from about 30% to 70% and from about 35% to

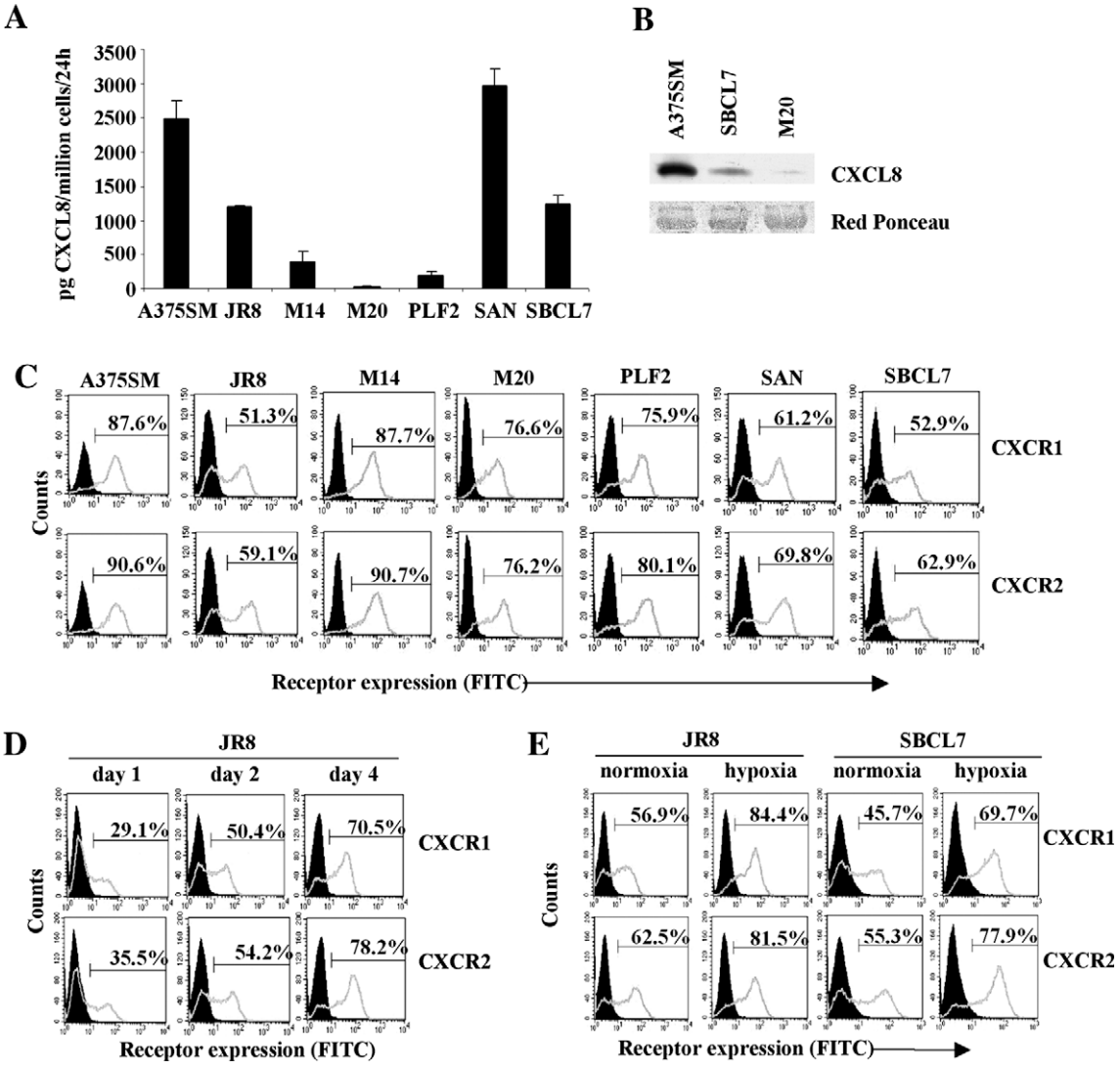

Fig. 1 – CXCL8 secretion and cell surface expression of CXCR1 and CXCR2 in human melanoma cell lines. Expression of CXCL8 in conditioned medium evaluated by ELISA (A) or Western blot (B). (C)–(E) Cytofluorimetric analysis of CXCR1 and CXCR2 surface expression in different melanoma lines. The percentage of positive cells is indicated. Black histograms: FITC-IgG2a isotype control.

78% for CXCR1 and CXCR2, respectively. As reported in Fig. 1E an enhanced expression of CXCR1 and CXCR2 was also observed after exposure of JR8 and SBCL7 cells to hypoxia, increasing the percentage of CXCR1 positive cells from about 57% to 84% for JR8 and from about 46% to 70% for SBCL7, and the percentage of CXCR2 positive cells from about 62% to 81% for JR8 and from about 55% to 78% for SBCL7.

Among the cell lines analysed, A375SM and M20, which showed the highest and the lowest levels of CXCL8 secretion, respectively, but similar cell surface expression of both CXCR1 and CXCR2, were used to evaluate the role of CXCL8 and its receptors in melanoma progression.

3.2. *Effect of CXCL8 on in vitro cell proliferation, invasion and migration*

We then evaluated the *in vitro* response to exogenous CXCL8 in terms of cell proliferation, invasion and migration (Figs. 2 and 3). As reported in Fig. 2, a dose-dependent enhancement of cell proliferation after treatment with rhCXCL8 was observed in A375SM and M20 cells grown in serum-containing or serum-free medium, respectively; an increase of cell proliferation of about 40% ($p < 0.001$) was induced by higher doses of rhCXCL8 in both cell lines. CXCL8 neutralising antibodies decreased cell proliferation of about 50% ($p < 0.001$) and 30% ($p < 0.001$) in unstimulated A375SM and rhCXCL8-stimulated M20 cells, respectively (data not shown). As expected,[28] the mitogenic response induced by rhCXCL8 was paralleled by AKT and ERK1/2 protein phosphorylation, which is indicative of PI3K and MAPK pathways activation (Fig. 2B). These results indicate an autocrine and a paracrine effect of CXCL8 on the proliferation of A375SM and M20 lines, respectively.

As reported in Fig. 3A, B and E a significant increase in migration in response to rhCXCL8 of A375SM (about 2-fold), M20 (about 3-fold), JR8 (about 1.5-fold) and SBCL7 (1.7-fold), was observed when compared to migration in response to PBS. Similarly, the ability to invade matrigel-coated filters was enhanced when A375SM (about 5-fold), M20 (about 5-fold), JR8 (about 2-fold) and SBCL7 (about 3.7-fold) lines were exposed to rhCXCL8 (Fig. 3C, D and F). Additional experiments were also performed on hypoxic melanoma cells in the presence of CXCL8. As reported in Fig. 3E and F, under hypoxic conditions, a significant increase in both migration and invasion in response to rhCXCL8 of JR8, (about 1.6- and 1.8-fold, respectively) and of SBCL7 (about 1.8- and 1.6-fold, respectively) cells, was observed when compared to migration and invasion under normoxic conditions. Random migration and invasion in response to PBS were not affected by hypoxic conditions in both cell lines (data not shown).

3.3. *Effect of antibodies neutralising CXCR1 and CXCR2 on in vitro cell proliferation, invasion and migration*

To investigate the role of CXCL8 receptors in proliferation, migration and invasion, A375SM and M20 cells were exposed to antibodies neutralising CXCR1 (anti-CXCR1) or CXCR2 (anti-CXCR2). As reported in Fig. 4A, both neutralising antibodies reduced the proliferation of A375SM and M20 lines; inhibition of about 35% ($p < 0.001$) and 25% ($p < 0.001$) induced by anti-CXCR1 was observed in the proliferation of both cell lines, respectively. Comparably, the exposure to anti-CXCR2 determined an approximate 25% inhibition of proliferation of A375SM ($p < 0.001$) and M20 ($p < 0.01$) cells. In addition, the exposure to both neutralising antibodies in combination significantly inhibited proliferation of A375SM cells when compared to the effect on proliferation of anti-CXCR1 ($p < 0.001$) or anti-CXCR2 ($p < 0.001$) alone. On the contrary, no significant differences were found when the effect on M20 cell proliferation of both CXCR1 and CXCR2 neutralising antibodies was compared to the effect obtained by exposing cells to each single antibody.

Moreover, a significant inhibition of cell migration (about 40%, Fig. 4B) and invasion (about 50%, Fig. 4C and D) was observed only when both cell lines were treated with anti-CXCR2, while no significant effect of anti-CXCR1 was observed on the migration and invasion of A375SM and M20 lines, and no differences were observed when the effect of the two antibodies given in combination was compared to the single administration of anti-CXCR2.

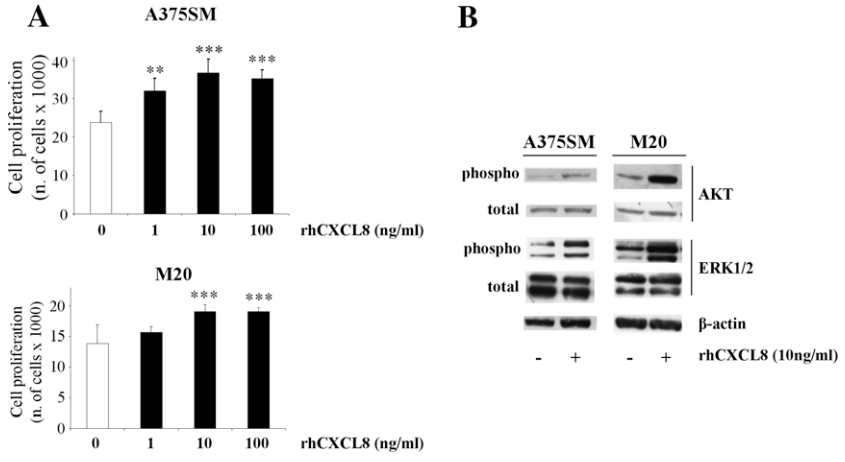

Fig. 2 – Effect of the exogenous CXCL8 on *in vitro* cell proliferation. Cell proliferation (A) and Western blot analyses of AKT and ERK1/2 expression and phosphorylation (B) in response to 72 h exposure to recombinant human CXCL8 (rhCXCL8).

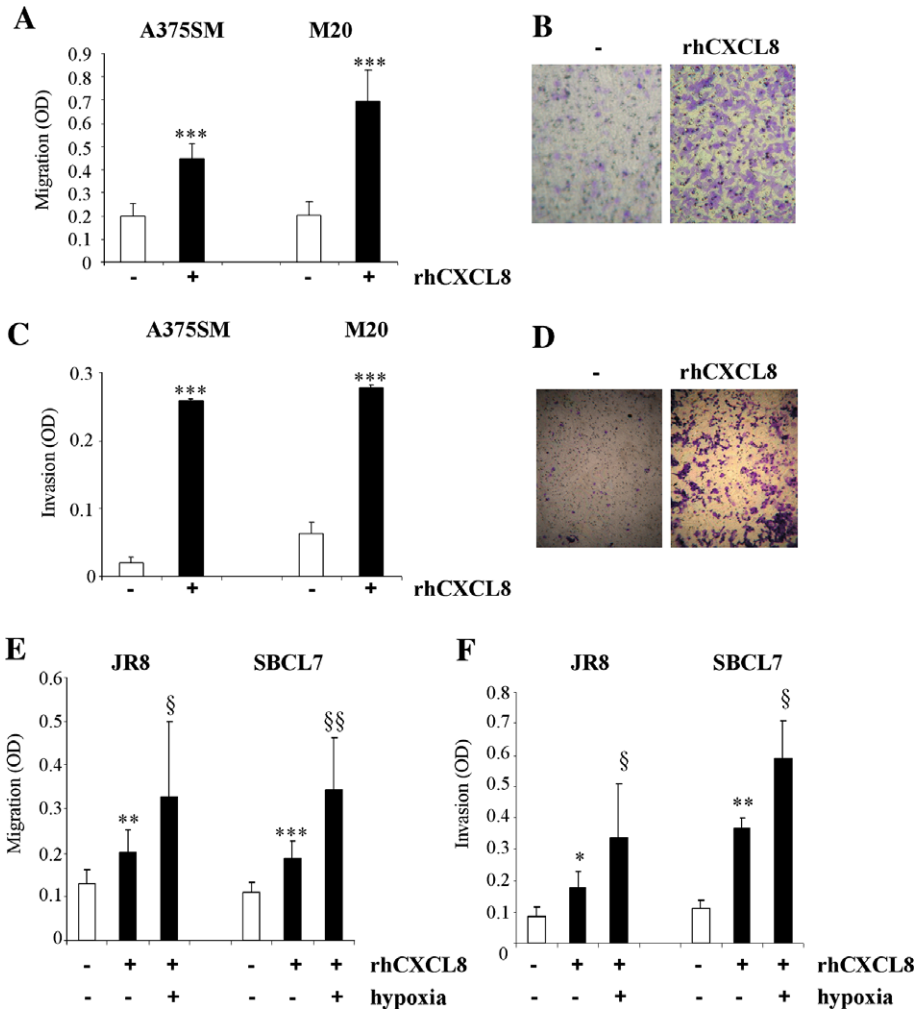

Fig. 3 – Effect of CXCL8 on cell migration and invasion. Migration (A, B and E) and invasion (C, D and F) in response to rhCXCL8 (10 ng/ml). Representative microphotographs of M20 cell migration (B) and invasion (D).

3.4. *Role of CXCL8 and its receptors on in vivo vessel formation*

Since CXCL8 is a potent inducer of angiogenesis,[12] the ability of A375SM and M20 cells to induce vessel formation and the relevance of CXCL8, CXCR1 and CXCR2 in this phenomenon were evaluated by an *in vivo* matrigel assay (Figs. 5 and 6). As evidenced by a macroscopic analysis of matrigel plugs, M20 cells induced a slight angiogenic response compared to A375SM cells, which showed a high angiogenic response (Fig. 5A). The quantification of the Hb content (Fig. 5B) and blood vessels (Figs. 5C and 6D) in the matrigel plugs evidenced about 5-fold increase in the Hb content and MVD in plugs containing A375SM cells when compared to plugs containing M20 cells.

By using antibodies neutralising CXCL8, CXCR1 or CXCR2 we investigated a possible role for CXCL8 and its receptors as mediators of the *in vivo* angiogenesis induced by A375SM cells (Fig. 6). As evidenced by macroscopic analysis (Fig. 6A), an approximate 3-fold decrease of Hb content (Fig. 6B, $p < 0.001$) and MVD (Fig. 6C and D, $p < 0.001$) was observed in matrigel plugs containing A375SM cells in the presence of antibodies neutralising CXCL8 when compared to plugs containing cells without the antibodies. Moreover, as reported by both macroscopic analyses (Fig. 6A) and Hb content (Fig. 6B), a significant reduction of vascularisation was observed after injecting matrigel plugs containing cells in the presence of anti-CXCR1 ($p < 0.05$) or anti-CXCR2 ($p < 0.001$) when compared to matrigel containing cells without any antibody. A similar significant reduction of MVD was observed in the presence of CXCR1 or CXCR2 neutralising antibodies ($p < 0.001$) (Fig. 6C and D). In addition, microscopic examination revealed the presence of a variable chronic inflammatory infiltrate, prevalently observed at the peripheral edge, in all matrigel plugs containing tumour cells, without significant differences among experimental groups (data not shown).

4. Discussion

Even though CXCL8 and its receptors have been suggested as key factors in melanoma progression,[1,7,11,17,29] the knowledge regarding the involvement of CXCR1 and CXCR2 in this phenomenon is poorly understood. In this study we evaluated the expression and the function of CXCL8 protein and its

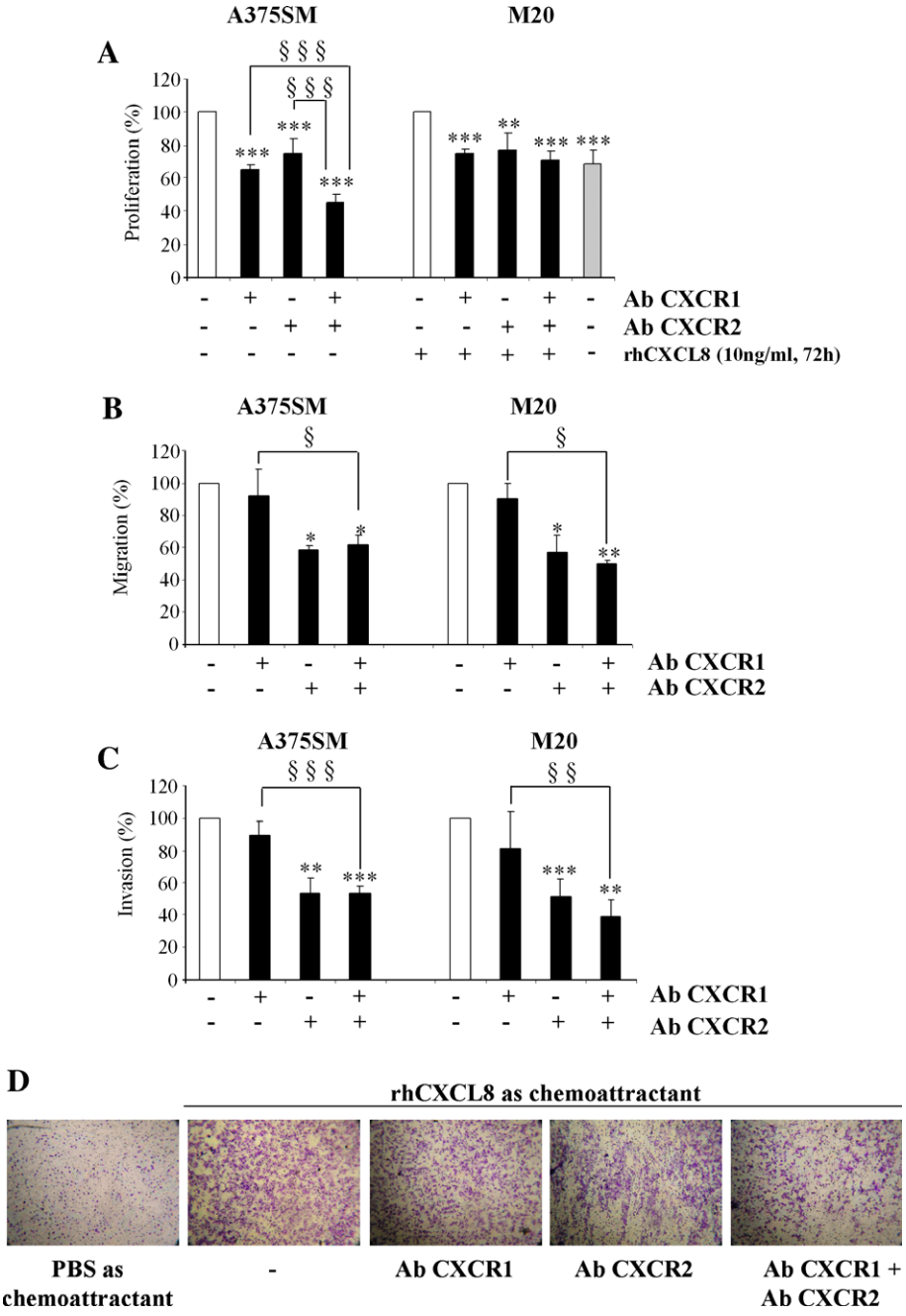

Fig. 4 – Effect of antibodies neutralising CXCR1 and CXCR2 on cell proliferation, migration and invasion. Proliferation (A), migration (B) and invasion (C) in the presence of antibodies neutralising CXCR1 (Ab CXCR1) and/or CXCR2 (Ab CXCR2). (A)–(C) *p* versus untreated cells (*) or versus cells exposed to one antibody alone (§). (D) Representative microphotographs of M20 cell invasion.

receptors on cell proliferation, migration, invasion and angiogenesis of human melanoma cell lines. We demonstrated that CXCL8 acts as autocrine and/or paracrine growth and pro-angiogenic factor for melanoma through CXCR1 and CXCR2, and it induces melanoma invasion, and migration through CXCR2, but not through CXCR1, demonstrating a different role of CXCR1 and CXCR2 in modulating an aggressive melanoma phenotype. Consistent with previous observations, detectable levels of CXCL8 protein and high cell surface expression of CXCR1 and CXCR2 were found in all the lines tested,[7,18] and the expression of the two receptors was independent from the level of CXCL8 secreted.[17] The expression of CXCR1 and CXCR2 increased during the cell growth *in vitro* and after exposure to hypoxia, a characteristic feature of solid tumour, in agreement with the finding of Hypoxia Responsive Element sequences on both CXCR1 and CXCR2 promoters,[2] and with the contribution of hypoxia to melanocyte transformation and melanoma development or aggres-

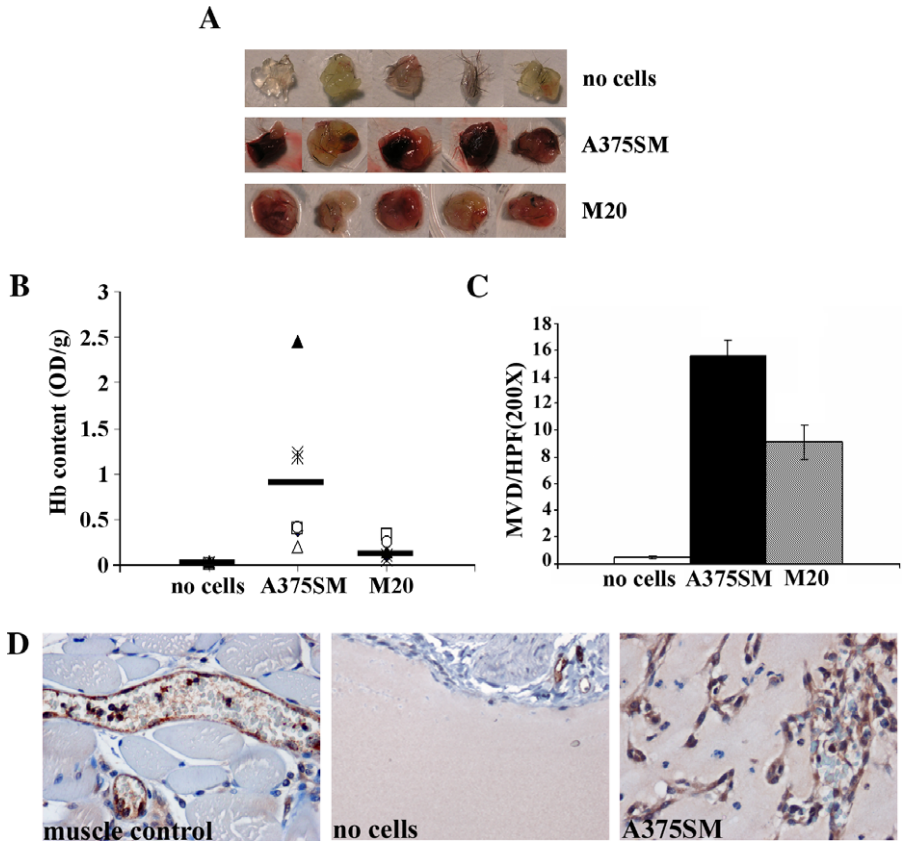

Fig. 5 – Evaluation of haemoglobin (Hb) content and microvessel density (MVD) in matrigel plugs. Macroscopic analysis (A), Hb content (B) and MVD (C) of matrigel plugs containing A375SM or M20 cells. (D) Representative immunostainings of Von Willebrand's factor-positive vessel endothelial cells in control skeletal muscle biopsy and section of matrigel plug containing A375SM cells.

siveness.[30,31] The induction of both receptors may give rise to enhanced paracrine CXCL8 signalling in hypoxic melanoma cells: the increased receptor pool could bind and sequester an increased amount of agonist, resulting in an acceleration of agonist-induced effects. To support this hypothesis our results demonstrated an increased response to CXCL8 under hypoxic conditions in terms of both cell migration and cell invasion.

Next we evaluated the involvement of CXCR1 and CXCR2 on *in vitro* and *in vivo* CXCL8-mediated functions. We observed an increased cell proliferation paralleled by ERK1,2 and AKT activation in response to rhCXCL8, indicating that CXCR1 and CXCR2 expressed on the surface of M20 and A375SM melanoma cells are functionally coupled to intracellular signalling events. In agreement with other authors,[13] we found that cell proliferation of both A375SM and M20 cells was significantly reduced in the presence of neutralising CXCL8 antibodies. We also showed that CXCL8 induces cell proliferation through both CXCR1 and CXCR2. The individual contribution of the receptors to cell proliferation was demonstrated using neutralising antibodies. Moreover, the cell proliferation inhibition determined by the combination of anti-CXCR1 with anti-CXCR2 is superimposable to that obtained after anti-CXCL8 treatment of both cell lines, suggesting that the downstream signalling pathways activated by these receptors cooperate to promote cell cycle progression.

In addition, the invasion and migration of A375SM and M20 cells were increased in response to CXCL8 in agreement with previous observations which suggested that CXCL8 may promote melanoma progression.[7,17,18] The use of specific neutralising antibodies also demonstrated that CXCL8-induced cell invasion and migration were through CXCR2, but not through CXCR1. The relevance of CXCR2 on *in vitro* cell migration and invasion was confirmed by simultaneous exposure of the cells to the two neutralising antibodies, in fact the addition of anti-CXCR1 did not increase inhibition induced by anti-CXCR2. The expression level of CXCR1 and CXCR2 may not account for these differences because in our study CXCR2 was expressed at similar levels as CXCR1.

Controversial papers have been published regarding the role of CXCR1 and CXCR2 on melanoma progression: while the involvement of both receptors in cell proliferation and invasive potential has been demonstrated by some authors,[17] only CXCR1 has been shown to be involved in CXCL8-induced chemotaxis,[10] besides a positive correlation between CXCR2, but not CXCR1, and growth,[18] Clark level and thickness[7] has been reported. In addition, the relevance of host CXCR2-dependent CXCL8-mediated angiogenesis in the regulation of melanoma growth and metastasis has also been reported.[32] Consistent with our results, invasion and chemotaxis of other tumour histotypes, such as prostate carcinoma, are mediated by CXCR2.[21] Also endothelial cell and fibroblast migrations

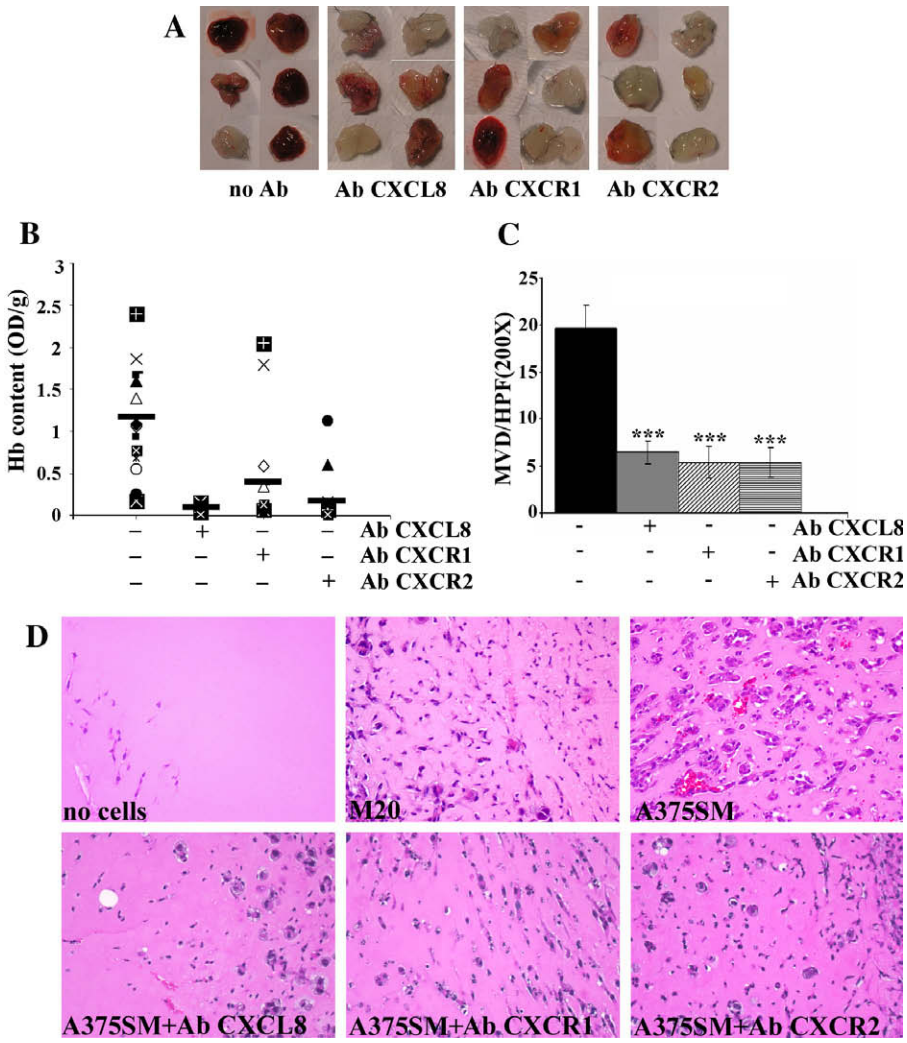

Fig. 6 – Effect of CXCL8, CXCR1 and CXCR2 neutralising antibodies on *in vivo* vessel formation. Macroscopic analysis (A), quantitative evaluation of haemoglobin (Hb) content (B) and microvessel density (MVD) (C) of matrigel plugs containing A375SM cells in the presence or absence of antibodies neutralising CXCL8 (Ab CXCL8), CXCR1 (Ab CXCR1) or CXCR2 (Ab CXCR2). (D) Representative images of Haematoxylin-Eosin-stained matrigel plugs.

are mostly mediated by CXCR2, despite the surface expression of CXCR1 and CXCR2 and similar binding affinity for CXCL8.[33,34] In other hands, neutrophil chemotaxis was shown to be mediated mainly via CXCR1, with minor contributions from CXCR2[11,35], and CXCR1 mediated CXCL8-induced colon carcinoma chemotaxis.[20] Thus, even when both receptors are present, distinct signalling pathways and cellular functions can be activated by the same ligand, depending on the cellular context. Clearly, the modulation of chemotaxis is a complex issue and data obtained from different chemokine receptors in different cell lines represent the complexity of chemotaxis in the mammalian system. Even though the precise mechanism by which CXCR1 and CXCR2 activate different signalling is not clear, the carboxy-terminus of each receptor seems to play a regulatory role, at least in leucocytes.[15]

Using an *in vivo* matrigel assay and neutralising antibodies we have also demonstrated that the angiogenic activity of melanoma cells is mediated by CXCL8, through both CXCR1 and CXCR2. We excluded a possible involvement of the pro-angiogenic vascular endothelial growth factor on the angiogenic potential because it was not detectable on the CM of both cell lines (data not shown).

In conclusion, our current observations provide further insight into the potential relevance of CXCL8 signalling through CXCR1 and CXCR2 in promoting melanoma progression. Whereas previous studies have suggested that CXCL8 neutralising antibodies inhibited *in vivo* tumour growth and artificial metastases of melanoma cells mainly through an antiangiogenic mechanism,[13] the data reported here support the concept that targeting CXCL8, CXCR1 and CXCR2 could reduce proliferation and angiogenesis of melanoma and that CXCR2 neutralisation could be protective against invasion and migration. Thus, blocking the CXCL8 signalling axis offers potential therapeutic promise for the therapy of cancer[15,18] and, in particular, of metastatic melanoma.[29]

Conflict of interest statement

None declared.

Acknowledgements

We thank Dr. Isaiah Fidler for providing A375SM cell line, Dr. Daniela Passeri for preparation of histological and immunohistochemical stainings, Dr. Adele Petricca for secretarial assistance and Paula Franke for the English revision of the manuscript.

REFERENCES

1. Schadendorf D, Moller A, Algermissen B, et al. IL-8 produced by human malignant melanoma cells in vitro is an essential autocrine growth factor. *J Immunol* 1993;**151**:2667–75.
2. Maxwell PJ, Gallagher R, Seaton A, et al. HIF-1 and NF-kappaB-mediated upregulation of CXCR1 and CXCR2 expression promotes cell survival in hypoxic prostate cancer cells. *Oncogene* 2007;**26**:7333–45.
3. Zigler M, Villares GJ, Lev DC, Melnikova VO, Bar-Eli M. Tumor immunotherapy in melanoma: strategies for overcoming mechanisms of resistance and escape. *Am J Clin Dermatol* 2008;**9**:307–11.
4. Leslie MC, Bar-Eli M. Regulation of gene expression in melanoma: new approaches for treatment. *J Cell Biochem* 2005;**94**:25–38.
5. Nurnberg W, Tobias D, Otto F, Henz BM, Schadendorf D. Expression of interleukin-8 detected by in situ hybridization correlates with worse prognosis in primary cutaneous melanoma. *J Pathol* 1999;**189**:546–51.
6. Ugurel S, Rappl G, Tilgen W, Reinhold U. Increased serum concentration of angiogenic factors in malignant melanoma patients correlates with tumor progression and survival. *J Clin Oncol* 2001;**19**:577–83.
7. Varney ML, Johansson SL, Singh RK. Distinct expression of CXCL8 and its receptors CXCR1 and CXCR2 and their association with vessel density and aggressiveness in malignant melanoma. *Am J Clin Pathol* 2006;**125**:209–16.
8. Giorgini S, Trisciuoglio D, Gabellini C, et al. Modulation of bcl-xL in tumor cells regulates angiogenesis through CXCL8 expression. *Mol Cancer Res* 2007;**5**:761–71.
9. Gabellini C, Castellini L, Trisciuoglio D, et al. Involvement of nuclear factor-kappa B in bcl-xL-induced interleukin 8 expression in glioblastoma. *J Neurochem* 2008;**107**:871–82.
10. Ramjeesingh R, Leung R, Siu CH. Interleukin-8 secreted by endothelial cells induces chemotaxis of melanoma cells through the chemokine receptor CXCR1. *FASEB J* 2003;**17**:1292–4.
11. Dong C, Slattery MJ, Liang S, Peng HH. Melanoma cell extravasation under flow conditions is modulated by leukocytes and endogenously produced interleukin 8. *Mol Cell Biomech* 2005;**2**:145–59.
12. Li A, Dubey S, Varney ML, Dave BJ, Singh RK. IL-8 directly enhanced endothelial cell survival, proliferation, and matrix metalloproteinases production and regulated angiogenesis. *J Immunol* 2003;**170**:3369–76.
13. Huang S, Mills L, Mian B, et al. Fully humanized neutralizing antibodies to interleukin-8 (ABX-IL8) inhibit angiogenesis, tumor growth, and metastasis of human melanoma. *Am J Pathol* 2002;**161**:125–34.
14. Cerretti DP, Kozlosky CJ, Vanden BT, et al. Molecular characterization of receptors for human interleukin-8, GRO/melanoma growth-stimulatory activity and neutrophil activating peptide-2. *Mol Immunol* 1993;**30**:359–67.
15. Richardson RM, Marjoram RJ, Barak LS, Snyderman R. Role of the cytoplasmic tails of CXCR1 and CXCR2 in mediating leukocyte migration, activation, and regulation. *J Immunol* 2003;**170**:2904–11.
16. Cataisson C, Ohman R, Patel G, et al. Inducible cutaneous inflammation reveals a protumorigenic role for keratinocyte CXCR2 in skin carcinogenesis. *Cancer Res* 2009;**69**:319–28.
17. Varney ML, Li A, Dave BJ, et al. Expression of CXCR1 and CXCR2 receptors in malignant melanoma with different metastatic potential and their role in interleukin-8 (CXCL-8)-mediated modulation of metastatic phenotype. *Clin Exp Metastasis* 2003;**20**:723–31.
18. Norgauer J, Metzner B, Schraufstatter I. Expression and growth-promoting function of the IL-8 receptor beta in human melanoma cells. *J Immunol* 1996;**156**:1132–7.
19. Murphy C, McGurk M, Pettigrew J, et al. Nonapical and cytoplasmic expression of interleukin-8, CXCR1, and CXCR2 correlates with cell proliferation and microvessel density in prostate cancer. *Clin Cancer Res* 2005;**11**:4117–27.
20. Bates RC, DeLeo III MJ, Mercurio AM. The epithelial-mesenchymal transition of colon carcinoma involves expression of IL-8 and CXCR-1-mediated chemotaxis. *Exp Cell Res* 2004;**299**:315–24.
21. Reiland J, Furcht LT, McCarthy JB. CXC-chemokines stimulate invasion and chemotaxis in prostate carcinoma cells through the CXCR2 receptor. *Prostate* 1999;**41**:78–88.
22. Trisciuoglio D, Iervolino A, Zupi G, Del Bufalo D. Involvement of PI3K and MAPK signaling in bcl-2-induced vascular endothelial growth factor expression in melanoma cells. *Mol Biol Cell* 2005;**16**:4153–62.
23. Trisciuoglio D, Desideri M, Ciuffreda L, et al. Bcl-2 overexpression in melanoma cells increases tumor progression-associated properties and in vivo tumor growth. *J Cell Physiol* 2005;**205**:414–21.
24. Del Bufalo D, Trisciuoglio D, Scarsella M, et al. Lonidamine causes inhibition of angiogenesis-related endothelial cell functions. *Neoplasia* 2004;**6**:513–22.
25. Del Bufalo D, Biroccio A, Leonetti C, Zupi G. Bcl-2 overexpression enhances the metastatic potential of a human breast cancer line. *FASEB J* 1997;**11**:947–53.
26. Orlandi A, Ciucci A, Ferlosio A, et al. Increased expression and activity of matrix metalloproteinases characterize embolic cardiac myxomas. *Am J Pathol* 2005;**166**:1619–28.
27. Weidner N, Semple JP, Welch WR, Folkman J. Tumor angiogenesis and metastasis – correlation in invasive breast carcinoma. *N Engl J Med* 1991;**324**:1–8.
28. Sai J, Walker G, Wikswo J, Richmond A. The IL sequence in the LLKIL motif in CXCR2 is required for full ligand-induced activation of Erk, Akt, and chemotaxis in HL60 cells. *J Biol Chem* 2006;**281**:35931–41.
29. Singh RK, Gutman M, Radinsky R, Bucana CD, Fidler IJ. Expression of interleukin 8 correlates with the metastatic potential of human melanoma cells in nude mice. *Cancer Res* 1994;**54**:3242–7.
30. Bedogni B, Powell MB. Hypoxia, melanocytes and melanoma – survival and tumor development in the permissive microenvironment of the skin. *Pigment Cell Melanoma Res* 2009;**22**:166–74.
31. Osawa T, Muramatsu M, Watanabe M, Shibuya M. Hypoxia and low-nutrition double stress induces aggressiveness in a murine model of melanoma. *Cancer Sci* 2009;**100**:844–51.
32. Singh S, Varney M, Singh RK. Host CXCR2-dependent regulation of melanoma growth, angiogenesis, and experimental lung metastasis. *Cancer Res* 2009;**69**:411–5.

33. Addison CL, Daniel TO, Burdick MD, et al. The CXC chemokine receptor 2, CXCR2, is the putative receptor for ELR+ CXC chemokine-induced angiogenic activity. *J Immunol* 2000;**165**:5269–77.
34. Burger M, Hartmann T, Burger JA, Schraufstatter I. KSHV-GPCR and CXCR2 transforming capacity and angiogenic responses are mediated through a JAK2-STAT3-dependent pathway. *Oncogene* 2005;**24**:2067–75.
35. Hammond ME, Lapointe GR, Feucht PH, et al. IL-8 induces neutrophil chemotaxis predominantly via type I IL-8 receptors. *J Immunol* 1995;**155**:1428–33.