Abstract. The placenta growth factor (PIGF) is a member of the vascular endothelial growth factor (VEGF) family, which shares with VEGF-A the tyrosine kinase receptor VEGFR-1 and the co-receptor neuropilin-1 (NRP-1). In melanoma models, PIGF enhances tumour growth and neovessel formation, whereas NRP-1 promotes the metastatic process. Increased secretion of PIGF and expression of NRP-1 have also been involved in intrinsic or acquired resistance to anti-angiogenic therapies. In this study we investigated whether PIGF and NRP-1 cooperate in promoting melanoma aggressiveness independently of VEGFR-1. For this purpose, the melanoma cell clones M14-N, expressing NRP-1 and lacking VEGFR-1, and M14-C, devoid of both receptors, were used. M14-N cells are characterized by an invasive phenotype and vasculogenic mimicry, whereas M14-C cells possess a negligible invasive capacity. The results indicated that M14-N cells secrete higher levels of PIGF than M14-C cells and that PIGF is involved in the invasion of the extracellular matrix (ECM) and vasculogenic mimicry of M14-N cells. In fact, neutralizing antibodies against PIGF reverted ECM invasion in response to PIGF and markedly reduced the formation of tubule-like structures. Moreover, M14-N cells migrated in response to PIGF and chemotaxis was specifically abrogated by anti-NRP-1 antibodies, demonstrating that PIGF directly activates NRP-1 in the absence of VEGFR-1. We also compared the levels of PIGF in the plasma of patients affected by metastatic melanoma with those of healthy donors and evaluated whether PIGF levels could be affected by a bevacizumab-containing chemotherapy regimen. Melanoma patients showed a 20-fold increase in plasma PIGF and the bevacizumab-containing regimen induced a reduction of VEGF-A and in a further increase of PIGF. In conclusion, our studies suggest that the activation of NRP-1 by PIGF directly contributes to melanoma aggressiveness and represents a potential compensatory pro-angiogenic mechanism that may contribute to the resistance to therapies targeting VEGF-A.

Introduction

Melanoma is a highly aggressive neoplasm, characterized by early metastatic spreading and poor response to chemotherapy and radiotherapy. Despite the recent advances in the treatment of the metastatic disease with the approval of monoclonal antibodies that block immune checkpoints (ipilimumab, nivolumab and pembrolizumab) and inhibitors of BRAF (venurafenib and dabrafenib) or MEK (trametinib), the prognosis of the advanced disease remains poor. Melanoma progression is favoured by the formation of new vessels from the pre-existing ones (angiogenesis), and by the ability of tumour cells to invade the extracellular matrix (ECM) and to form structures similar to blood vessels (vasculogenic mimicry).

A key role in the angiogenic process is played by members of the vascular endothelial growth factor (VEGF) family, and in particular by the VEGF-A and the placenta growth factor (PIGF) (1,2), which can be released by the tumour cell itself. VEGF-A interacts with two membrane tyrosine kinase receptors (3): VEGFR-1 (involved in the activation of cell migration and in the modulation of the signalling transduced by the VEGFR-2) and VEGFR-2 (mainly responsible for the signal transduction that mediates the effects of VEGF-A). Conversely, PIGF binds only to VEGFR-1, promoting migration, proliferation and survival of endothelial cells.

The expression of PIGF is upregulated in several types of human cancers and is associated with a poor prognosis (4,5). PIGF is capable of transducing its own signals through the phosphorylation of tyrosine residues within VEGFR-1, which are distinct from those phosphorylated upon stimulation of the receptor by VEGF-A (6). PIGF expression in the skin of
transgenic mice inoculated with syngeneic melanoma cells, favours tumour growth and mobilization of endothelial and hematopoietic stem cells. In the same murine model PIGF also increases the number and size of melanoma-associated vessels and the formation of pulmonary metastases (7). Moreover, PIGF promotes tumour cell invasion of the ECM and enhances the activity of selected matrix metalloproteinases (7). Interestingly, PIGF plays also a role in melanoma cell resistance to chemotherapy through a pathway that involves NF-κB activation (8).

The VEGFR-1 is expressed in endothelial cells during vessel formation and remodelling, in macrophages and in myoepithelial cells, favouring cell migration and survival (9-11). Moreover, it is frequently expressed in a variety of human tumours where it predicts poor prognosis and recurrence (1,9). In cancer cells, VEGFR-1 signalling has been shown to inhibit apoptosis and to induce chemoresistance (1,12,13). Indeed, VEGFR-1 neutralization has been reported to prolong survival of tumour-bearing mice (14). We previously demonstrated that PIGF and VEGFR-1 are co-expressed in a large number of human melanoma cell lines (15) and, together with other groups, suggested that the interaction of PIGF with VEGFR-1 might modulate cellular pathways important for melanoma cell proliferation, apoptosis and invasiveness (7,15-18).

Another receptor, capable of binding VEGF-A and PIGF is neuropilin-1 (NRP-1), a membrane protein devoid of tyrosine kinase activity. Through the interaction with VEGF-A, NRP-1 acts as a co-receptor, enhancing the signal transmitted by VEGFR-1 and VEGFR-2 (2,4,19-21). Moreover, although the intracellular domain of NRP-1 is not able to directly transmit a signal, its interaction with intracellular regulators allows NRP-1 to activate signal transduction pathways independently of tyrosine kinase VEGF-A receptors (22). In this regard, PIGF has been recently shown to activate MAPK and AKT signalling and promote tumour cell survival by interacting with NRP-1 in the absence of VEGFR-1 (23). Actually, a wide variety of human malignancies (cell lines and tissue samples) express NRP-1 and experimental evidence indicates an association between the expression of NRP-1 and tumour growth, invasiveness, angiogenesis and poor prognosis (24).

Given the key role played by VEGFs and VEGFRs in the process of angiogenesis, intensive preclinical and clinical investigation has been focused on the development of targeted drugs that interfere with their activation or signal transduction (25). To date, two angiogenic monoclonal antibodies have been approved for cancer treatment: bevacizumab, a human antibody against VEGFR-2 (C17) and anti-human NRP-1 mouse monoclonal antibody (A-12), the rabbit polyclonal antibodies anti-human VEGFR-1 (C17) and anti-human β-tubulin (H-235, used as a loading control) were purchased from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA, USA) and utilized at the final concentration of 0.2 μg/ml.

Migration and invasion assays. In vitro migration assays were performed using Boyden chambers equipped with 8-µm pore diameter polycarbonate filters (Nuclepore; Whatman Inc., Clifton, NJ, USA), coated with 5 μg/ml gelatin (30). Briefly, melanoma cells were collected from continuous cultures, washed, suspended in migration medium (1 μg/ml heparin/0.1% BSA in RPMI-1640) and loaded (1.5x10³ cells) into the upper compartment of the Boyden chambers. Migration medium, with or without 50 ng/ml PIGF, was added to the lower compartment and the chambers were incubated at 37°C in a CO₂ atmosphere for 18 h. The filters were then removed from
the chambers and cells were fixed in ethanol and stained in crystal violet. The migrated cells, attached to the lower surface of the filters, were counted under the microscope. Twelve high-magnification microscopic fields (magnification, x100), randomly selected on triplicate filters, were scored for each experimental condition.

In vitro ECM invasion by melanoma cells was analysed in Boyden chambers as described for the migration assay, except that polycarbonate filters were coated with 20 µg of the commercial basement membrane matrix Matrigel (Trevigen, Gaithersburg, MD, USA) (18) and 1x10⁵ cells/chamber were allowed to invade for 2 h.

In selected experiments, migration or invasion assays were performed in the presence of antibodies against PlGF (mouse monoclonal anti-human antibody, MAB264 from R&D Systems, Inc.), NRP-1 (rabbit polyclonal anti-human antibody, H286 from Santa Cruz Biotechnology, Inc.), and VEGFR-1 (goat polyclonal anti-human antibody; AF321) and VEGFR-2 (goat polyclonal anti-human antibody; AF357) (both from R&D Systems, Inc.) or the corresponding control immunoglobulins (mouse IgG1 isotype control from R&D systems, Inc.). Melanoma cells were pre-incubated with the antibody under investigation for 30 min at room temperature in a rotating wheel and, then, loaded in the Boyden chambers.

ELISA quantification of VEGF and PlGF levels. For the evaluation of PlGF secretion by melanoma cell lines, semi-confluent cells were incubated for 24 h in 0.1% BSA/RPMI-1640 and VEGF levels were determined using commercial ELISA kits (R&D Systems, Inc.), following the instructions from the manufacturer.

Differentiation of melanoma cells in tubule-like structures. The analysis of tubule-like structures on Matrigel was performed as previously described (29). Melanoma cells were suspended in complete culture medium (1x10⁵ cells in 0.5 ml), dispensed onto 100 µl of solidified Matrigel (diluted 1:3 in serum-free RPMI-1640 medium) and incubated for 6 h at 37°C in a 5% CO₂ environment. Afterwards, the plates were photographed using a Leica inverted microscope equipped with a Canon digital camera (PowerShot G5). The formation of tubule-like structures was quantified by counting the number of cell intersections in ten different microscopic fields for each experimental group (x50 magnification).

Studies in patients with metastatic melanoma. Plasma levels of VEGF-A and PlGF were measured in eight healthy donors and eight melanoma patients enrolled in a phase II study evaluating bevacizumab (10 mg/kg days 1 and 15, q28) in combination with paclitaxel (60 mg/m², days 1, 8, and 15, q28) and carboplatin (AUC 2, days 1, 8 and 15, q28), as a second-line treatment for metastatic melanoma. The study protocol was approved by the Ethics Committee of the 'Istituto Dermopatico dell’Immacolata' - IRCCS, at the 02/12/08 meeting (Protocol number #133/EC/2008, Reference number: Register of the Ethics Committee 247/1; Promoter: IDI-IRCCS, Oncology Division, Professor Marchetti; EudraCT code: 2008-06191-30; Protocol: IDI-ONC-3-20080901). Written informed consent was obtained from all the subjects (patients and controls).

The inclusion criteria were as follows: age between 18 and 75 years; histologically confirmed metastatic melanoma progressing after one line of chemotherapy not containing platinum analogues; measurable metastatic disease according to the Response Evaluation Criteria in Solid Tumours (RECIST); ECOG PS ≤2; life expectancy of more than 3 months; adequate blood parameters (leukocytes >4,000/mm³, neutrophils >2,000/mm³, platelets >100,000/mm³, haemoglobin >10 g/dl, serum creatine <2.5 mg/dl, total bilirubin/GOT/GPT <1.5x the upper limit).

The exclusion criteria were as follows: presence of brain metastases, a history of other cancers diagnosed within 5 years (except basal cell skin cancer or carcinoma in situ of the cervix); previous treatment with platinum analogues and/or bevacizumab; concomitant immunotherapy; radiation therapy performed within 28 days before treatment; a history of myocardial infarction, congestive heart failure or heart disease and/or uncontrolled, active infections; acute and/or chronic HIV, hepatitis and/or tuberculosis infections; peripheral neuropathy (higher than grade 2, according to the NCIC-CTC criteria); contraindication to the use of corticosteroids (unstable diabetes mellitus, active stomach ulcer); baseline LDh level >1.5x the upper limit of the normal range; pregnancy or breast feeding.

Blood samples were collected during the first cycle of therapy on day 1 before bevacizumab and chemotherapy administration, on day 8 before chemotherapy administration and on day 15 before bevacizumab and chemotherapy administration.

Results

NRP-1 enhances PlGF-mediated ECM invasion in melanoma cells expressing VEGFR-1. We have previously demonstrated in a murine model that PlGF promotes the invasiveness of melanoma cells through the activation of its tyrosine kinase receptor VEGFR-1 (7). In order to investigate the requirement of NRP-1 for this PlGF function, we initially evaluated the ability of PlGF to stimulate ECM invasion using melanoma cell lines that we had previously characterized for VEGFR-1 and NRP-1 expression by RT-PCR analysis (15) (Fig. 1). M14 cells, lacking VEGFR-1 and expressing very low levels of NRP-1, and GL-Mel cells, expressing VEGFR-1 but lacking NRP-1, did not respond to PlGF. Conversely, WM266-4 cells, expressing both VEGFR-1 and NRP-1, efficiently migrated through the ECM when exposed to the growth factor. In these cells a complete abrogation of melanoma cell invasiveness was observed when the assay was carried out in the presence of a blocking antibody against VEGFR-1, whereas a blocking
antibody against VEGFR-2, used as a control, did not have any effect on PIGF-induced invasion (Fig. 1). These results suggested that PI GF strongly induced ECM invasion only when melanoma cells expressed high levels of NRP-1, besides VEGFR-1.

In VEGFR-1 negative melanoma cells PI GF promotes ECM invasion and vasculogenic mimicry through the interaction with NRP-1. We then investigated whether NRP-1 acts only as co-receptor for VEGFR-1 or is also able to initiate by itself a signal transduction after PI GF binding in the absence of VEGFR-1. To this end, we used two previously described cell clones derived from the M14 melanoma cell line: the M14-N cells, characterized by an aggressive phenotype, and the M14-C cells with a negligible invasive capability (28,31). The M14-N cells lack VEGFR-1 and express high levels of NRP-1 protein, whereas the M14-C cells do not express either VEGFR-1 or NRP-1 (Fig. 2A). Evaluation of PI GF secretion in the culture supernatants indicated an increased secretion of this cytokine by M14-N cells as compared to M14-C cells (Fig. 2B). Moreover, PI GF specifically induced ECM invasion by M14-N cells, since an anti-PI GF antibody completely reversed this effect, whereas an unrelated control antibody did not affect the ECM invasion triggered by this cytokine (Fig. 2C and D).

The invasiveness of M14-N cells is strongly hampered by NRP-1 neutralization (28), making it difficult to demonstrate a direct activation of NRP-1 by PI GF. Therefore, the ability of PI GF to activate NRP-1 in M14-N cells was tested using a chemotaxis assay, in which the background migration was very low and the stimulation by this cytokine strongly induced migration (Fig. 3A and B). It should be noted that due to the highly invasive phenotype of M14-N cells, for the invasion assay a short incubation time was sufficient (2 h), whereas the migration assay required a longer incubation (18 h). Blocking antibodies to PI GF or to the NRP-1 were equally able to completely abrogate PI GF-induced chemotaxis.

A characteristic of highly aggressive cancers, and in particular of melanoma (32), is the ability to form tubule-like structures (vasculogenic mimicry), which provide neoplastic cells with nutrients and facilitate their haematogenous spreading. M14-N cells are able to form tubular structures and this property is strongly dependent on NRP-1 activity (29). Herein we demonstrate that PI GF is directly involved in the formation of tubule-like structures (Fig. 3C and D). In fact, an anti-PI GF blocking antibody markedly reduced cell-to-cell intersections, which were instead unaffected by the treatment with a control antibody (Fig. 3C and D). Thus, the effects of PI GF on vasculogenic mimicry of M14-N cells likely require the activation of NRP-1.

All these data strongly suggested the presence of an autocrine PI GF/NRP-1 loop in highly aggressive melanoma cells.

Analysis of plasma levels of PI GF and VEGF-A in patients with metastatic melanoma. We next investigated whether high PI GF levels could be present in the plasma of patients with metastatic melanoma, and whether the level of this cytokine could be affected by a chemotherapy regimen including bevacizumab to target angiogenesis. To this end, we took advantage from a phase II study carried out to evaluate bevacizumab in combination with carboplatin and paclitaxel as a second-line therapy in patients with metastatic melanoma.

From March 2009 to May 2011, 51 patients with metastatic melanoma progressing after a first line of chemotherapy were examined for eligibility. On the basis of the exclusion criteria, 42 patients (21 with secondary brain injury; 10 with ECOG PS ≥3; 8 with no measurable target lesions; 2 with previous heart attack and 1 with previous ictus cerebri) were not enrolled in the study. In addition, one patient withdrew informed consent at the time of the first drug administration. Therefore, only eight patients, five males and three females with a median age of 56 years (38-66), were available for the analysis of VEGF-A and PI GF levels in the blood.

Plasma levels of VEGF-A and PI GF in the eight melanoma patients were determined on day 1, 8 and 15 of the first cycle of therapy. Blood samples were collected just before the administration of the drugs. VEGF-A and PI GF levels detected in the plasma collected from patients on day 1 (i.e., baseline values) were compared with those of age- and gender-matched healthy donors. The results showed significant higher amounts of VEGF-A and PI GF in the patients as compared to the healthy controls. Remarkably, we observed a 20-fold difference in the levels of PI GF between melanoma patients and healthy volunteers (Fig. 4).

For each of the eight patients, VEGF-A and PI GF plasma levels detected on day 8 and 15 were then compared with baseline values (day 1). The results indicated that after a single administration of bevacizumab plus chemotherapy, VEGF-A markedly decreased (day 8) and started to increase again on day 15, even though its levels remained significantly below the values detected before starting the treatment cycle (Fig. 5). On
the other hand, plasma PlGF significantly increased on day 8 (2.3-fold increase) and remained high until the end of the cycle (3-fold increase) (day 15; Fig. 5).

Overall, the results indicated that patients with metastatic melanoma show measurable levels of PlGF in the plasma and that treatment with bevacizumab induces a further increase of this cytokine.

Discussion

In the present study we demonstrate for the first time that PlGF promotes ECM invasion and vasculogenic mimicry of human melanoma cells through the activation of NRP-1 and independently of VEGFR-1. These findings strongly suggest a role for PlGF and NRP-1 in melanoma aggressiveness and in the activation of compensatory pro-angiogenic mechanisms that may contribute to innate or acquired resistance to anti-angiogenic therapies targeting VEGF-A.

The involvement of PlGF in melanoma progression has been previously suggested (7,15-18,33) and confirmed by studies performed in a murine transgenic model where high PlGF expression levels were selectively induced in the skin (7). On the other hand, NRP-1, initially considered only a co-receptor of tyrosine kinase receptors for different ligands, has been more recently shown to play an important role in the activation of specific signal transduction pathways independently of other membrane receptors (23,34-36). Our previous data indicate that NRP-1, upon interaction with VEGF-A, may contribute to the melanoma metastatic potential even in the absence of VEGFR-2, stimulating tumour invasiveness and vasculogenic mimicry, which facilitates the haematogenous spreading of cancer cells (28,29). These effects require the triggering of signal transduction pathways involving AKT (28), specific gene expression (31), integrin activation (29) and the secretion of metalloproteinases that degrade the ECM, such as metalloproteinases 2 and 9 (28,29,37).

Even though disease progression is highly dependent on angiogenesis, melanoma may undergo phenotypic changes which limit the efficacy of anti-VEGF-A agents. Here we demonstrate that in melanoma cells NRP-1 may be activated by PlGF also in the absence of VEGFR-1. Both PlGF and NRP-1 are, therefore, involved in the induction of mechanisms that may result in tumour progression and resistance to anti-angiogenic therapies.
Figure 3. PlGF induces a chemotactic response in M14-N cells through the activation of NRP-1 and is involved in vasculogenic mimicry. (A) The chemotactic response of M14-N cells to PlGF was evaluated using Boyden chambers equipped with gelatine-coated filters. Representative microscopic fields (x200 magnification) are shown that correspond to: non-stimulated cells (a); cells stimulated with 50 ng/ml PlGF in the absence of antibodies (Ab) (b); cells stimulated with PlGF in the presence of 5 µg/ml mouse monoclonal anti-PlGF Ab (c); cells stimulated with PlGF in the presence of 5 µg/ml rabbit polyclonal anti-NRP-1 Ab (d); and cells stimulated with PlGF in the presence of 5 µg/ml control Ab (e). (B) The results of a representative experiment out of three are shown. Data represent the mean number of cells per microscopic field ± SD of 12 fields in triplicate chambers for each experimental condition. (C) Effect of the mouse monoclonal Ab against PlGF on the ability of M14-N cells to form tubule-like structures on Matrigel. Cells were untreated (a) or pre-incubated with 5 µg/ml of anti-PlGF Ab (b) or control Ab (c) for 30 min in a rotating wheel at room temperature, before seeding them on Matrigel. Tubule-like structure formation was analyzed after 6 h. Photographs from a representative experiment out of three are shown (x50 magnification). (D) The number of cell intersections formed by the tubule-like structures obtained in the experiment shown in panel C was counted in ten different microscopic fields for each experimental group. Data represent the mean ± SD of a representative experiment. **P<0.01 and ***P<0.001, according to Student's t-test analysis.

Figure 4. Evaluation of the plasma levels of VEGF-A and PlGF in healthy donors and melanoma patients. Box and whisker plots for the levels of VEGF-A and PlGF in the plasma of healthy donors (n=8) and melanoma patients (n=8), as determined by ELISA. In melanoma patients, blood samples were withdrawn on day 1 of the first cycle of a bevacizumab/paclitaxel/carboplatin regimen, just before drug administration. The top and bottom of each box represent the 75th and 25th percentile, respectively, and whiskers the 10th and 90th percentiles. The horizontal bar within each box indicates the median. Data were analysed by ANOVA for multiple comparisons followed by Bonferroni test; *P-value <0.05.
In our melanoma model, we found that cells expressing NRP-1 produce a higher amount of PlGF as compared to the NRP-1 negative cells. PlGF is directly involved in the invasive phenotype of M14-N cells, since neutralizing antibodies reverted ECM invasion in response to the growth factor. Moreover, the chemotactic response of NRP-1 positive cells to exogenous PlGF was abrogated by anti-NRP-1 antibodies, suggesting that a direct interaction of PlGF with NRP-1 is required for PlGF-mediated melanoma invasiveness. Thus, in VEGFR-1 negative cells NRP-1 behaves as a primary receptor capable of transmitting the signal deriving from its binding to PlGF.

Noteworthy, we also found increased levels of PlGF in the plasma of patients with metastatic melanoma as compared to healthy donors. These patients were enrolled in a phase II clinical trial in which bevacizumab was administered in combination with the chemotherapeutic agents paclitaxel and carboplatin. Drug treatment was followed by a transient decrease of VEGF-A and a parallel increase of PlGF plasma levels. In patients with non-small cell lung cancer, treated with a bevacizumab-carboplatin-paclitaxel regimen, a decrease in VEGF-A was also reported and associated with clinical benefit (38). Due to the small number of melanoma patients enrolled in the clinical study, we cannot draw any definitive conclusion about the predictive value of VEGF-A decline and PlGF increase in the plasma of patients. Nevertheless, our results encourage further studies in a larger number of patients to evaluate the role of plasma PlGF as a potential marker of melanoma resistance to anti-VEGF-A treatment.

High levels of PlGF can result in tumour progression through autocrine or paracrine mechanisms. PlGF can be produced not only by tumour cells but also by cells of the tumour stroma (39). Indeed, in a murine in vivo model, the increased plasma levels of PlGF in response to an anti-angiogenic therapy were shown to derive from the host tissues and not from the tumour itself (40). Moreover, using human melanoma cell lines secreting both VEGF and PlGF, we found that treatment with bevacizumab did not cause any modulation of PlGF release in the culture supernatants (data not shown). Therefore, in patients treated with an anti-VEGF-A therapy high plasma levels of PlGF, produced by the tumour or by the stroma, can favour melanoma spreading as long as cancer cells express a significant amount of NRP-1, even in the absence of VEGFR-1.

Considerable experimental evidence allows hypothesizing a potential role for PlGF or NRP-1 as therapeutic targets. Differently from VEGF-A, PlGF plays a marginal role in physiological conditions in adults, but is involved in the induction of the angiogenic switch in pathological conditions, in the mobilization of hematopoietic precursor stem cells from the bone marrow and in the growth and migration of cancer cells (41). Moreover, in preclinical animal models monoclonal antibodies specific for PlGF inhibit the growth and metastasis in different types of tumours and increase the efficacy of chemotherapy without causing significant side effects (4,6,41).

Concerning NRP-1 as therapeutic target, this receptor is expressed in endothelial, highly aggressive melanoma and immune cells and, therefore, is involved in all the biological processes crucial for melanoma progression: angiogenesis, vasculogenic mimicry and tumour evasion from the control of the immune system. Moreover, a high-affinity monoclonal antibody targeting NRP-1 has been shown to inhibit migration of human endothelial cells and tumour formation in animal models (42,43). It also inhibited breast cancer cell proliferation and enhanced chemosensitivity of human non-small cell lung, kidney, prostate cancer and other carcinoma cells, by interfering with integrin-dependent survival pathways (44-46). Thus, the targeting of NRP-1 seems to be a valuable strategy for combination therapies, including anti-angiogenic agents, as reviewed (47).

In conclusion, in this study we have shown that PlGF promotes ECM invasiveness and vasculogenic mimicry of melanoma cells, and that these functions are principally mediated by PlGF activation of NRP-1. Moreover, we found that the plasma levels of PlGF in melanoma patients are higher...
than in healthy donors, and that these levels tend to further increase in the course of a bevacizumab-containing chemotherapy regimen. PlGF production and NRP-1 expression may, therefore, increase tumour aggressiveness and contribute to melanoma escape from anti-VEGF-A therapies. Thus, the blockade of PlGF interaction with its receptors, VEGFR-1 and, more importantly, NRP-1, represents a therapeutic strategy that may enhance the efficacy or counteract resistance to inhibitors of VEGF-A mediated pathways.

Acknowledgements

This study was supported by the Italian Ministry of Health and in part by the ‘Associazione Italiana per la Ricerca sul Cancro’ (AIRC) to GG (AIRC 2013 IG 14042).

References