available at www.sciencedirect.com

SCIENCE DIRECT®

journal homepage: www.ejconline.com

Potential role of CCL27 and CCR10 expression in melanoma progression and immune escape

Oriana Simonetti[a,*], *Gaia Goteri*[b], *Guendalina Lucarini*[c], *Alessandra Filosa*[b], *Tiziana Pieramici*[b], *Corrado Rubini*[b], *Graziella Biagini*[c], *Annamaria Offidani*[a]

[a]*Clinica Dermatologica, Università Politecnica delle Marche, Ospedali Riuniti, Via Conca 71, 60020 Torrette (Ancona), Italy*
[b]*Istituto di Patologia, Università Politecnica delle Marche, Ancona, Italy*
[c]*Istituto di Morfologia-Istologia Umana Normale, Università Politecnica delle Marche, Ancona, Italy*

ARTICLE INFO

Article history:
Received 13 October 2005
Received in revised form
12 January 2006
Accepted 16 January 2006
Available online 27 April 2006

Keywords:
Melanoma
Immunohistochemistry
CCL27
CCR10
Host immune response

ABSTRACT

Aim of this study was to investigate using immunohistochemistry techniques the interrelation between T immunoreactive cells and the expression of CCR10 and its ligand CCL27 in 59 cutaneous melanocytic lesions. In malignant melanomas, T lymphocyte density was significantly decreased from thin melanomas to intermediate and thick ones ($P < 0.0005$). CCR10 expression was found both in benign and malignant lesions and it was directly correlated with the Breslow depth ($P = 0.0298$) and inversely with T lymphocyte density ($P = 0.0231$). Moreover, cases with positive sentinel lymph node tended to have a higher CCR10 expression compared to cases with negative sentinel lymph node ($P = 0.0281$). When CCR10 and CCL27 expression were evaluated together, CCR10-/CCL27-melanomas tended to have a higher mean density of CD3+ and CD8+ lymphocytes. Our results suggest that in human melanomas CCR10 and CCL27 may act to increase the ability of neoplastic cells to grow, invade tissue, disseminate to lymph nodes and to escape the host immune response.

1. Introduction

Cutaneous melanoma is a tumour generated from activated or genetically modified melanocytes[1,2] with a rapidly increasing incidence in the white population. Malignant melanoma may arise de novo or, less frequently, from potential precursor lesions, such as atypical dysplastic nevi or congenital nevi.[3,4] The ability of tumour cells to avoid immune surveillance represents, among other factors, a critical step in tumour progression.[5] Tumour immunology embraces an extensive array of biological phenomena that include interactions between neoplastic cells and the innate and adaptive immune response. It has been suggested that the expression of chemokines and chemokine receptors by melanoma cells may be involved in tumour immune escape.[6] Chemokines are a large family of structurally related, small polypeptide signalling molecules that regulate leukocyte-migration and invasion into tissues by interacting with their specific receptors, which belong to the superfamily of seven-transmenbrane domain G-protein-coupled receptors.[7] Although chemokines are classically regarded as proinflammatory mediators, they also exhibit a number of other well characterised functions, having a role in normal tissue development,[8] angiogenesis[9] and control of hemopoietic stem and progenitor cell proliferation.[10] They are also involved in cancers in the cellular neoplastic transformation, growth, invasion and homing of metastasis to distant sites,[11] and in the host anti-tumour response.[5] It has been

* *Corresponding author:* Tel.: +39 071 5963494; fax: +39 071 5963446.
E-mail address: o.simonetti@univpm.it (O. Simonetti).
0959-8049/$ - see front matter
doi:10.1016/j.ejca.2006.01.043

hypothesized that migration of tumour cells in specific organs may be determined by the chemokine receptors the cells express and by the chemokines expressed in the target organs.[6] Specifically, melanoma cells were found to express high levels of CXCR4, CCR7 and CCR10 mRNA when compared with normal melanocytes.[5] Moreover, investigators demonstrated in murine melanoma that B16 cells overexpressing CCR10 resisted host immune responses and readily formed tumours.[12] CCR10 is a specific receptor of the skin-related chemokine CCL27 found to be a selective chemo-attractant for cutaneous-associated Ag-positive T and Langerhans cells[13] and could play a role in T cell homing in inflammed skin.[14,15] The role of the CCR10/CCL27 interaction in melanomas is unclear at present. It is possible to suppose that CCL27 in melanomas can induce antitumoural immunity, as described by Guo et al. in a murine lung carcinoma model, where CCL27-mediated tumour regression was obtained by an efficient induction of antitumour immunity[16]: CCL27 could suppress tumour growth via transfection into the tumour cells probably due to the local recruitment of T cells and natural killer cells.[17] However, recent publications on murine melanoma have suggested that liganding of tumour-associated CCR10 with CCL27 may be a mechanism to promote cancer cell survival and reduce immune response in animal models.[12]

Data from the literature prompted us to investigate the interrelation between T immunoreactive cells and the expression of CCR10 and its ligand CCL27 in human melanoma tissues, by analysing the possible variations also in relation to tumour thickness, invasion levels and lymph node metastasis, and by comparing the results obtained in melanomas with a selected sample of benign melanocytic lesions.

2. Materials and methods

Fifty-nine cases of cutaneous melanocytic lesions included in this study were obtained from the Archives of the Institute of Pathology of Ancona University. Biopsy specimens obtained at surgery were fixed in 10% buffered formalin and embedded in paraffin. Diagnoses were made on haematoxylin and eosin-stained sections, but Fontana-Masson staining and immunoistochemical staining with S-100, NSE and HMB-45 antibodies was also performed in selected cases. Diagnoses were based on the usual criteria.[18] The formalin fixed, paraffin embedded melanocytic lesions represented 40 primary cutaneous human melanomas (17 males; 23 females) with invasion (Clark's levels II–III, 16 cases; levels IV–V, 24 cases); 15 had a Breslow thickness $\leqslant$1.0 mm; 12 of 1.1–4.0 mm; 13 of >4.1 mm. None of them presented regression areas. Twenty-four patients underwent examination of sentinel lymph node: except for one patient who had a thin melanoma, all the others had a thick (11 cases) or an intermediate melanoma (12 cases). Ten out of 24 patients (42%) were positive for metastasis (6 females, 4 males; 6 intermediate and 4 thick melanomas). Nineteen benign lesions were also retrieved, 10 spindle and epithelioid cell Spitz nevi, and 9 ordinary benign melanocytic nevi, either compound or predominantly intradermal.

2.1. Immunohistochemistry

Regarding immunohistochemistry, conventional 6 μm-thick histological sections were obtained with a microtome from the selected blocks and mounted on slides pre-treated with poly-L-lysine (Sigma Chemicals, St. Louis, MO, USA). The slides were then deparaffined and rehydrated in a gradient of ethanol and xylene. Antigenic retrieval was performed with two different methods: sections were incubated with a solution of EDTA 0.01 M at pH 8.0 at 95–98 °C for 30 min before staining with anti-CD3, -CD4 and -CD8, and with a solution of TUF (Target Unmasking Fluid; Alexis, Biochemical) at 90 °C for 10 min before staining with anti-CCR10 and CCL27. After washing in H_2O and then in Tris buffer solution, the sections were incubated with the following monoclonal antibodies: mouse anti-CD4 (1F6, dil. 1:40, Novocastra Laboratories Ltd., Newcastle, UK), mouse anti-CD8 (4B11, dil. 1:40, Novocastra Laboratories Ltd., Newcastle, UK), rabbit anti-CD3 (dil. 1:100, Dakocytomation, Milano, Italy), mouse anti-CCL27 (dil. 1:200, R&D Systems, Minneapolis, MN, USA) and polyclonal antibody anti-CCR10 (dil. 1:500 Alexis-Italia, Vinci, Firenze, Italy). Incubations with the antibodies were carried out in a humidified atmosphere at room temperature for 1 h for anti-CD3, -CD4, -CD8 and overnight at 4 °C for anti-CCL27 and anti-CCR10. The antibodies were linked to an alkaline phosphatase anti-alkaline phosphatase (APAAP) catalysed reaction (Kit Dako, SpA, Italy) being washed roughly with PBS between all stages in the procedure. For visualization of the antibody sections were incubated with the substrate Fast red (Dakocytomation, Italy), yielding a red end product at the site of the target antigen. The sections were finally counterstained with Mayer's haematoxylin, dehydrated and mounted in glycerol-gelatin (Dakocytomation, Italy). Each series included appropriate negative control sections omitting the primary antibody reaction.

T lymphocyte subsets were evaluated with a light microscope by scanning the entire melanocytic lesions: intralesional and peri-lesional positive lymphocytes were counted together and their amount was expressed as number of positive cells per high power field (HPF).

Immunostainings for CCR10 and CCL27 were also evaluated with a light microscope by scanning the entire melanocytic lesions: epidermal and dermal positive melanocytes were counted together and their amount was expressed as percentage of positive cells.

2.2. Statistical analysis

Statistical analysis was performed in order to compare the CCR10 and CCL27 expression in melanocytes in different histologic lesions (benign nevi, Spitz nevi, malignant melanomas); in particular, in melanomas these variables were correlated with CD3+, CD4+ and CD8+ T lymphocyte density and analysed with respect to biologic characteristics of the tumours, like the depth of infiltration, measured on histologic slides, Clark's level and metastasis in sentinel lymph node. For statistical analysis, two categories of Clark's level were considered by grouping together cases with Clark's level II and III and cases with level IV and V; cases with Breslow depth <1.0 mm were grouped in the category of thin melanomas,

cases with Breslow thickness >1.1 and <4.0 mm were grouped in the category of intermediate melanomas, whereas cases with Breslow thickness >4.0 were considered thick melanomas.

Data were summarised by mean and range for continuous variables. Differences between mean values in different categories were analysed by unpaired Student *t* test and non-parametric Kruskal–Wallis test. Correlations between continuous variables were analysed by means of the Spearman correlation test. All statistical analysis was performed by SPSS statistical package (SPPS Inc. Chicago, IL).

3. Results

Data regarding the density of T lymphocytes and helper or suppressor subsets in the different diagnostic categories are reported in Table 1.

3.1. *T lymphocyte density*

T lymphocyte density was correlated with the histologic type of lesions. It was indeed found higher in malignant melanomas compared to benign nevi and Spitz nevi (Kruskal–Wallis test: $P < 0.0005$). The increase of T lymphocytes was not limited to a particular subset of T lymphocytes, because both CD4+ and CD8+ cells showed the same trend (Kruskal–Wallis test: $P < 0.0005$ for both). In malignant melanomas, T lymphocyte density was found inversely correlated to the depth of infiltration ($r = -0.596$, $P < 0.0001$): melanomas cases with II–III Clark level showed a higher T lymphocyte mean density compared to cases with IV–V Clark's level (unpaired *t* test: $P < 0.0001$). T lymphocyte density was significantly lower in thick melanomas compared to thin and intermediate melanomas (Kruskal–Wallis test, $P < 0.0005$). T lymphocyte density did not correlate with metastasis in sentinel lymph node (unpaired *t* test, $P = 0.96$).

3.2. *CCR10 and CCL27 immunostaining*

Data regarding melanocytic expression and intensity of the immunostaining and number of positive cells for CCR10 and CCL27 in the different diagnostic categories, are reported respectively in Tables 2 and 3.

CCR10 immunostaining was not found in 7 cases (1 benign nevus, 1 Spitz nevus and 5 melanomas). The other cases showed a variable cytoplasmic staining, ranging from moderate

Table 1 – CD3+, CD4+, CD8+ lymphocytes density in benign nevi and malignant melanomas

Histology	Cases (number of cases)	Lymphocyte density (number of cells/HPF) mean ± SD					
		CD4+	*P*	CD8+	*P*	CD3+	*P*
Melanocytic lesion	[a]Benign nevi (9)	1.9 ± 2.9		6.1 ± 4.2		8.1 ± 5.9	
	[b]Spitz'nevi (10)	7.9 ± 5.9		9.1 ± 7.9		17.0 ± 12.3	
	[c]Melanomas (40)	13.6 ± 11.8	<0.0005* (vs. a and b)	17.2 ± 12.3	<0.0005* (vs. a and b)	31.0 ± 20.6	<0.0005* (vs. a and b)
Clark's level	[a]II–III (16)	21.2 ± 12.9	<0.0001** (vs. b)	24.4 ± 14.2	<0.0001** (vs. b)	45.6 ± 20.1	<0.0001** (vs. b)
	[b]IV–V (24)	7.9 ± 6.8		12.3 ± 7.6		20.2 ± 13.3	
Breslow	[a]<1.0 mm (15)	20.9 ± 13.3		23.1 ± 13.9		44.0 ± 19.9	
	[b]>1.0 and <4 mm (12)	12.9 ± 8.0		18.2 ± 10.6		30.0 ± 17.1	
	[c]>4.0 mm (13)	4.4 ± 4.0	<0.0005* (vs. a and b)	9.5 ± 6.9	<0.0005* (vs. a and b)	13.6 ± 10.2	<0.0005* (vs. a and b)
Sentinel lymph node	[a]Negative (44)	8.2 ± 7.3		13.7 ± 8.1		21.9 ± 14.1	
	[b]Positive (10)	10.1 ± 8.3	NS**	15.1 ± 11.8	NS**	25.5 ± 20.0	NS**

SD: standard deviation; HPF: high power field; NS: not significant.
* Kruskal–Wallis test.
** Unpaired *t* test.

Table 2 – Intensity of CCR10 and CCL27 melanocytic expression in benign nevi and malignant melanomas

Histological cases (number of cases)	CCR10+			CCL27+		
	Intensity of immunostaining (% of cases)			Intensity of immunostaining (% of cases)		
	Negative	+/++	+++	Negative	+/++	+++
Benign nevi (9)	11.1	44.4	44.4	100	0	0
Spitz' nevi (10)	10	40	50	90	10	0
Melanomas (40)	12.5	17.5	70	55	40	5

+/++: staining intensity ranging from moderate to focal; +++: staining intensity strong and diffuse.

Table 3 – CCR10+ and CCL27+ melanocytic expression in benign nevi and malignant melanomas

Histology	CCR10+	CCL27+
	% Positive cells (mean ± SD)	% Positive cells (mean ± SD)
Benign nevi	42.8 ± 30.1	0.31 ± 0.6
Spitz' nevi	56.9 ± 28.9	4.9 ± 10.7
Melanomas	62.0 ± 33.5	12.6 ± 20.6
II–III Clark's level	51.5 ± 37.4	7.6 ± 10.4
IV–V Clark's level	69.0 ± 29.3	16.0 ± 24.9
Breslow <1.0 mm	46.7 ± 36.8	7.6 ± 10.7
Breslow >1.0 and <4.0 mm	61.9 ± 34.8	17.2 ± 31.6
Breslow >4.0 mm	79.7 ± 17.8	14.1 ± 16.6
Negative sentinel lymph node	58.1 ± 33.7	14.4 ± 23.4
Positive sentinel lymph node	84.1 ± 10.1	18.8 ± 27.5

SD: standard deviation.

and focal in 15 cases (4 benign nevi, 4 Spitz nevi and 7 melanomas), to strong and diffuse in 37 (4 benign nevi, 5 Spitz and 28 melanomas; Fig. 1a and b). The percentage of expression did not differ between nevi, Spitz nevi and melanomas (Kruskal–Wallis test, $P = 0.17$). However, in melanomas with metastatic sentinel lymph node, mean CCR10 expression was

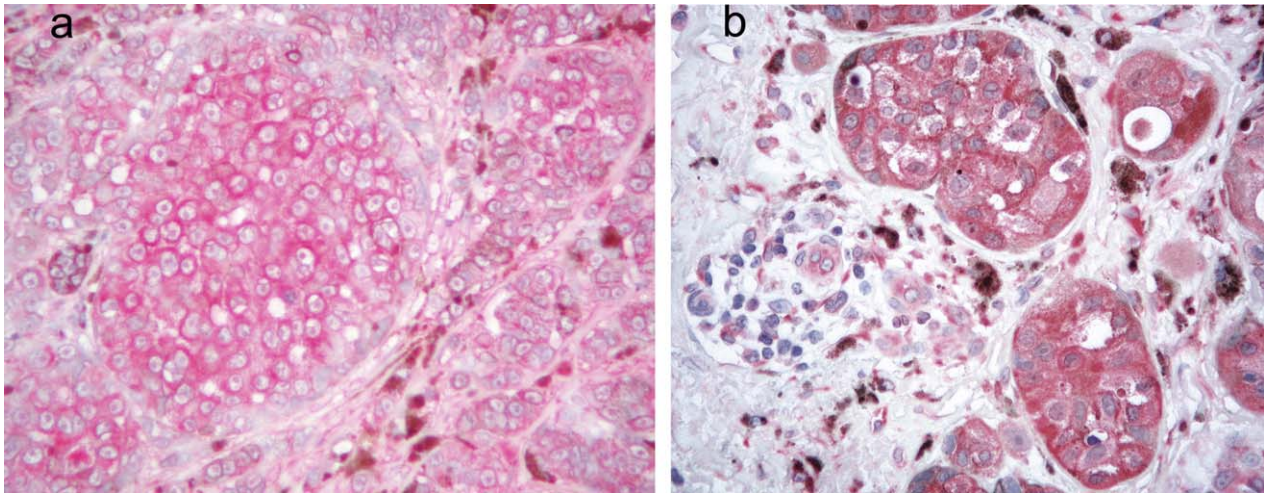

Fig. 1 – Strong and diffuse immunohistochemical expression of CCR10 in (a) thick and in (b) thin melanomas (APAAP, ×400).

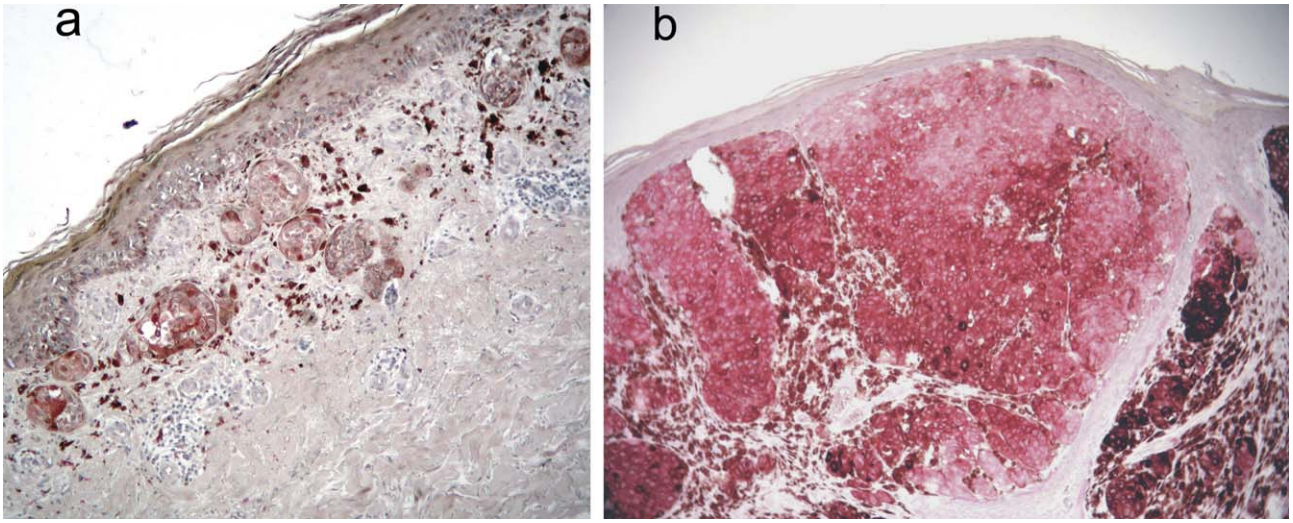

Fig. 2 – (a) Immunohistochemical CCL27 staining appears moderate (thin melanoma, APAAP ×200) and/or (b) strong (intermediate melanoma, APAAP ×100) in melanomas.

significantly higher than cases with negative lymph node (84.1% vs. 58.1% in metastatic cases; unpaired *t* test, $P = 0.0281$). Moreover, CCR10 expression in malignant melanocytes was correlated directly with the Breslow depth ($r = 0.343$; $P = 0.0298$) and inversely with T lymphocyte density ($r = -0.363$; $P = 0.0231$).

CCL27 was expressed in the cytoplasms of epidermal keratinocytes in all lesions. In all benign lesions, CCL27 was negative or faintly expressed by single melanocytes, except in one case of Spitz nevus, which showed a moderate and focal expression and a high T cell density. In melanomas CCL27 was negative in 22 cases, weakly to moderately and focally expressed in 16 (Fig. 2a), and strongly and diffusely expressed in 2 (Fig. 2b). CCL27 expression in malignant melanocytes was also found to be inversely correlated with T lymphocyte density ($r = -0.333$; $P = 0.0381$).

Ten cases of melanomas expressed both CCR10 and CCL27 in more than 20% of the cells (+/+ phenotype), 23 cases expressed only CCR10 in more than 20% of the cells (+/− phenotype), whereas 7 cases were negative or expressed both antigens in less than 20% of the cells (−/− phenotype). Cases with a double negative phenotype tended to have a higher mean density of CD3+ (47.16/HPF; Fig. 3) and CD8+ lymphocytes (24.89/HPF; Fig. 4) compared to cases showing expression of CCR10 alone (CD3+: 31.51/HPF, Fig. 3; CD8+: 18.72/HPF, Fig. 4) or both CCR10 and CCL27 (CD3+: 18.01/HPF, Fig. 3; CD8+: 8.87/HPF, Fig. 4). CD3+ and CD8+ lymphocytes mean densities were statistically significant between the three groups considered above (Kruskal–Wallis for CD3+ density: $P = 0.0154$;: for CD8+ density: $P = 0.009$). Cases with a double negative phenotype tended to have a lower tumour thickness (0.90 mm) compared to cases showing expression

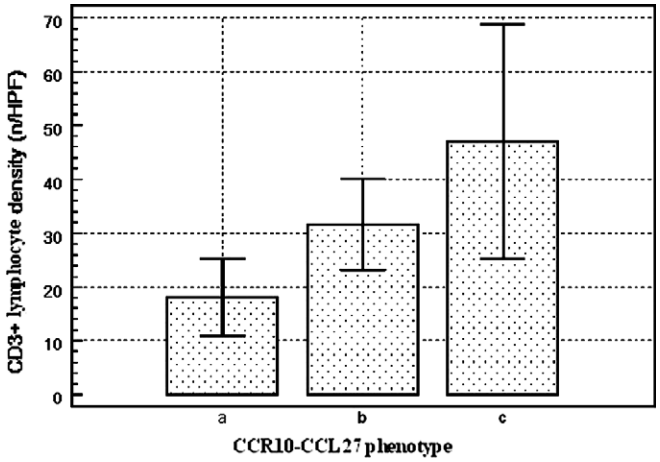

Fig. 3 – CD3+ T lymphocyte density in relation to the expression of CCR10 and CCL27 in malignant melanocytes. Ten cases of melanomas expressed both CCR10 and CCL27 in more than 20% of the cells (+/+ phenotype), 23 cases expressed only CCR10 in more than 20% of the cells (+/− phenotype), whereas 7 cases were negative or expressed both antigens in less than 20% of the cells (−/− phenotype). Cases with a double negative phenotype tended to have a higher mean density of CD3+ (c: 47.16/HPF) compared to cases showing expression of CCR10 alone (b:31.51/HPF) or CCR10 and CCL27 together (a: 18.01/HPF).

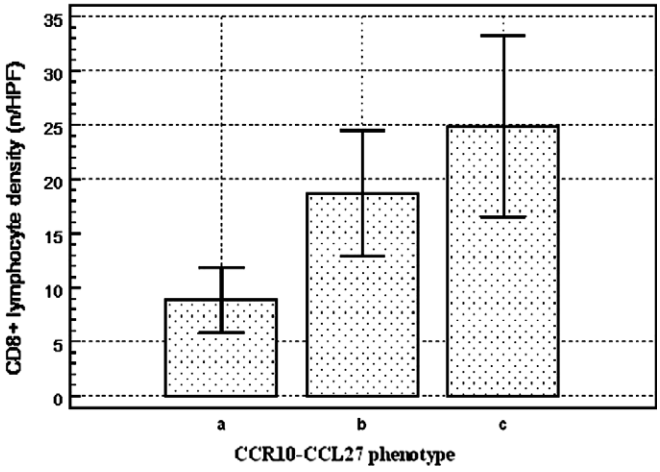

Fig. 4 – CD8+ T lymphocyte density in relation to the expression of CCR10 and CCL27 in malignant melanocytes. Cases with a double negative phenotype tended to have a higher mean density of CD8+ lymphocytes (c: 24.89/HPF) compared to cases showing expression of CCR10 alone (b: 18.72/HPF) or both CCR10 and CCL27 (a: 8.87/HPF).

of CCR10 alone (3.17 mm) or both CCR10 and CCL27 (3.95 mm Kruskal–Wallis: $P = 0.13$).

4. Discussion

Melanoma is considered an immunogenic tumour because of the identification of numerous tumour-associated antigens,[19] the well-known occurrence of spontaneous regression mediated by the host immune response[20] and the detection of antigen-experienced anti-tumour specific T lymphocytes in vivo.[21] The presence of a lymphocytes infiltrate in the tumour has been included amongst the prognostic factors for many years, especially in cases with vertical growth.[22,23] Although, recent cumulative data indicate that the presence of clonally expanded T cells in melanomas is not necessarily associated with an effective anti-tumour immune response and may represent subsets with different functions. In fact tumoural lymphoid population is composed not only of CD8+ cytotoxic lymphocytes or $CD4^+/CD25^-$ cells, specific for tumoural antigens, but also of regulatory T cells with a $CD4^+/CD25^{high}$ phenotype and inhibiting local antitumour response.[24,25] In our study on melanomas with a wide range of level of infiltration and with both horizontal and vertical growth, T lymphocyte density is confirmed to be inversely correlated to tumour thickness, similar to observations of other investigators,[26] indicating that melanomas with a greater thickness show a reduction of immune competent cells regardless of lymphocyte specific function and phenotype. Our intention was to study furthermore the T cell populations in relation to the interaction between CCL27 and CCR10. Our results seem to suggest that the aggressive behaviour and the immune escape in melanomas could involve CCR10 together with its ligand CCL27. CCR10 immunopositivity of melanocytes was present both in benign (89%) and malignant (84%) melanocytic lesions, although in our series 70% of CCR10+ melanomas showed strong immunostaining compared to 47% of CCR10+ benign nevi. From our results it can be inferred that malignant neoplastic cells are characterised by a CCR10 overexpression. CCR10 expression is induced in melanoma cells by TNF-α and IL-1β,[13] and a number of studies have shown that melanoma can produce several growth factors and cytokines, including IL-1β and TNF-α[27,28]; therefore, these molecules may lead to CCR10 overexpression in melanoma cells. In melanomas we observed a direct correlation of CCR10 with tumour thickness and an inverse correlation with T lymphocytic density. Moreover we observed a trend for cases with positive sentinel lymph node to have a higher expression of CCR10, although the number of cases that underwent this procedure seems too scant to establish a clear-cut correlation. It follows that CCR10 expression might be related to an aggressive behaviour of melanoma and to its ability to escape immune control. Experiments on animals have already shown that CCR10 expression in B16 melanoma cells is associated with a striking increase in metastasis to the draining lymph node after injection into the footpads of mice.[12] In human malignant melanomas, about 60% of metastases occur in regional lymph nodes,[29] thus lymph node metastasis status represent an important predictor of survival for this disease.[23] An important role in determining the aggressive behaviour and the immune escape of melanomas can also be played by the CCR10-ligand CCL27. We found CCL27 expression in the cytoplasm not only of basal keratinocytes, but also of malignant melanocytes, in 45% of melanoma cases. Benign melanocytes from nevi were all negative or faintly CCL27 positive, except one case of Spitz. CCL27, produced in the epidermis and then secreted into the papillary dermis, could be immobilized on the extracellular matrix and subsequently displayed in the cytoplasm of melanocytic cells following transcytosis. This mechanism has been observed to occur normally in endothelial cells of superficial dermal plexus by Homey and colleagues[14] and probably requires CCR10 expression. In fact Gortz and colleagues[30] showed that exogenous mature CCL27 may be able to enter target cells by interacting with its receptor, CCR10, in a paracrine manner. Indeed, our samples of melanoma negative for CCR10 receptor, did not even show CCL27 chemokine immunostaining. The capability to internalize the CCL27 chemokine might be a feature of malignant melanocyte, because it was observed more frequently in melanomas than in benign lesions. We observed that melanoma cases positive for CCL27 tended to have a lower mean density of CD3+ and CD8+ compared to melanoma cases not expressing CCL27, suggesting that liganding of CCR10 by CCL27 could make melanoma cells less susceptible to the host antitumour response, in agreement with the results obtained by Murakami and colleagues in animal models.[12] Moreover, cases with CCR10 and CCL27 co-expression tended to have a higher tumour thickness, suggesting that liganding of CCR10 by CCL27 in melanoma cells could be associated with an advantage in tumour growth.[12] Since CCL27 can be regarded as a cellular response that recruit lymphocytes to the tumour site, the CCL27 internalization in neoplastic melanocytes expressing CCR10, by the CCL27/CCR10 interaction, could lead to a sequestering of the chemokine as an attempt to withdraw lymphocyte recruitment. CCL27 internalization in malignant cells can mediate a number of biological effects, such as an increase of migratory competence by inducing actin cytoskeleton relaxation, as recently reported.[30]

In conclusion, our preliminary results suggest that also in human melanomas CCR10 may act in increasing the ability of neoplastic cells to grow, invade tissue, disseminate to lymph nodes and to escape the host immune response. We suggest that co-expression of CCL27 and CCR10 by neoplastic cells could be linked with poorer prognosis in melanoma patients, although this hypothesis should be confirmed by studies performed in larger cohorts of patients. Moreover, a better understanding of the CCR10/CCL27 interaction in melanomas could have future potential therapeutic applications by inhibiting the function of the chemokine and its receptor and subsequently decreasing the ability of neoplastic cells to grow, disseminate to other organs and modulate the host immune response mediated by T lymphocytes against the tumour cells.

Conflict of interest statement

None declared.

REFERENCES

1. Nordlund JJ, Boissy RE, Hearing VJ, et al., editors. *The pigmentary system: physiology and pathophysiology*. New York (NY): Oxford University Press; 1988.
2. Lejeune FJ, Chaudhuri PK, Das Gupta PK, editors. *Malignant melanoma and surgical management*. New York (NY): McGrow-Hill; 1994.
3. Clark W, Elder DE, Guerry D, et al. A study of tumour progression: the precursor lesions of superficial spreading and nodular melanoma. *Hum Pathol* 1984;**15**:1147–65.
4. Herlin M, Clark WH, Rodeck U, et al. Biology of disease: biology of tumour progression in human melanocytes. *Lab Invest* 1987;**56**:461–74.
5. Payne AS, Cornelius LA. The role of chemokines in melanoma tumour growth and metastasis. *J Invest Dermatol* 2002;**118**:915–22.
6. Muller A, Homey B, Soto H, et al. Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001;**410**:50–6.
7. Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. *Immunity* 2000;**12**:121–7.
8. Nagasawa T, Hirota S, Tachibana K, et al. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature* 1996;**382**:635–81.
9. Strieter RM, DiGiovine B, Polverini S, et al. C–X–C chemokines and lung cancer angiogenesis. In: Rollins BJ, editor. *Chemokines and cancer*. Clifton (NJ): Humana; 1999. p. 143.
10. Graham GJ. Growth inhibitors in haemopoiesis and leukemogenesis. *Ballieres Clin Haematol* 1997;**10**:539–59.
11. Strieter RM, Polverini S, Kunkel SL, et al. The functional role of ELR motif in C–X–C chemokine mediated angiogenesis. *J Biol Chem* 1995;**270**:1083–6.
12. Murakami T, Cardones AR, Finkelstein SE, et al. Immune evasion by murine melanoma mediated through CC chemokine receptor-10. *J Exp Med* 2003;**198**:1337–47.
13. Homey B, Wang W, Soto H, et al. Cutting edge: the orphan chemokine receptor G protein-coupled receptor-2 (GPR-2, CCR10) binds the skin associated chemokine CCL27 (CTAK/ALP/ILC). *J Immunol* 2000;**164**:3465–70.
14. Homey B, Alenius H, Muller A, et al. CCL27–CCR10 interactions regulate T cell mediated skin inflammation. *Nat Med* 2002;**8**:157–65.
15. Morales J, Homey B, Vicari AP, et al. CTHCK, a skin associated chemokine that preferentially attracts skin homing-memory T cells. *Proc Natl Acad Sci USA* 1999;**96**:14470–5.
16. Guo J, Wang B, Zhang M, et al. Macrophage-derived chemokine gene transfer results in tumour regression in murine lung carcinoma model through efficient induction of antitumour immunity. *Gene Ther* 2002;**9**:793–803.
17. Okada N, Gao J-Q, Sasaki A, et al. Anti-tumour activity of chemokine is affected by both kinds of tumours and the activation state of the host's immune system: implications for chemokine-based cancer immunotherapy. *Biochem Biophys Res Commun* 2004;**317**:68–76.
18. Lever WF, Schaumburg-Lever G. *Histopathology of the skin*. 7th ed. Philadelphia (PA): J.B. Lippincott; 1990. p. 756–805.
19. Boon T, Coulie PG, Van den Eynde B. Tumour antigens recognized by T cells. *Immunol Today* 1997;**18**:267–8.
20. Nathanson L. Spontaneous regression of malignant melanoma: a review of the literature on incidence, clinical features, and possible mechanisms. *Natl Cancer Inst Monogr* 1976;**44**:67–76.
21. Romero P, Dunbar PR, Valmori D, et al. Ex vivo staining of metastatic lymph nodes by class I major histocompatibility complex tetramers reveals high number of antigen experienced tumour-specific cytolytic T lymphocytes. *J Exp Med* 1998;**188**:1641–50.
22. Clemente CG, Mihm MC, Bufalino R, et al. Prognostic value of tumour infiltrating lymphocytes in the vertical growth phase of primary cutaneous melanoma. *Cancer* 1996;**77**:1303–10.
23. Clark Jr WH, Elder DE, Guerry IV DP, et al. Model predicting survival in stage I melanoma, based on tumour progression. *J Natl Cancer Inst* 1989;**81**:1893–904.
24. Viguier M, Lemaitre F, Verola O, et al. Foxp3 expressing $CD4^{+}/CD25^{high}$ regulatory T cells are overpresented in human metastatic melanoma lymph nodes and inhibit the function of infiltrating T cells. *J Immunol* 2004;**173**:1444–53.
25. Bernsen MR, Dipestra JHS, van Mil P, et al. Presence and localization of T-cell subsets in relation to melanocyte differentiation antigen expression and tumour regression as assessed by immunohistochemistry and molecular analysis of microdissected T cells. *J Pathol* 2004; **202**:70–9.
26. Lyle S, Salhany KE, Elder DE. TIA-1 positive tumour-infiltrating lymphocytes in nevi and melanomas. *Mod Pathol* 2000;**13**:52–5.
27. Lazár-Molnár E, Hegyesi H, Tóth S, et al. Autocrine and paracrine regulation by cytokines and growth factors in melanoma. *Cytokine* 2000;**12**:547–54.
28. Slominski A, Wortsman J, Carlson AJ, et al. Malignant melanoma. *Arch Pathol Lab Med* 2001;**125**(10):1295–306.
29. McCarthy WH, Shaw HM, Thompson JF, et al. Time and frequency of recurrence of cutaneous stage I malignant melanoma with guidelines for follow-up study. *Surg Gynecol Obstet* 1988;**166**:497–502.
30. Gortz A, Nibbs RJB, McLean P, et al. The chemokine ESkine/CCL27 displays novel modes of intracrine and paracrine function. *J Immunol* 2002;**169**:1387–94.