PERGAMON

European Journal of Cancer 38 (2002) 1708–1716

European Journal of Cancer

www.ejconline.com

Overexpression of the cell adhesion molecule L1 is associated with metastasis in cutaneous malignant melanoma

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Received 24 September 2001; received in revised form 20 February 2002; accepted 19 March 2002

Abstract

Modulation of cell adhesion molecule expression plays a key role in melanoma metastasis. In particular, the expression of the cell adhesion molecule L1 has been associated with the metastatic phenotype in a murine model of malignant melanoma. However, no such association between L1 expression and metastasis has been investigated in a clinical study. Therefore, L1 expression was determined immunohistochemically in 100 cases of malignant melanoma and correlated with metastasis in a 10-year retrospective study. Furthermore, nine distant metastases and five sentinel lymph node metastases were analysed for their L1 expression. Additionally, the expression of α2,3 sialic acid residues, which are recognised by the siglec domain of L1, was determined by *Maackia amurensis* agglutinin (MAA) lectin histochemistry. The log-rank test between Kaplan–Meier curves revealed a positive association between L1 expression and metastasis ($P < 0.0001$) and multivariate Cox regression analysis adjusted for tumour thickness, ulceration and mitotic rate confirmed the prognostic power of L1 in malignant melanoma. As α2,3 sialic acid residues were absent in melanoma cells, homotypic adhesion between melanoma cells via their siglec domain can be excluded, suggesting a different adhesive function of L1 during melanoma metastasis. The functional role of L1 was further stressed by the fact that its expression was preserved in metastatic lesions.

Keywords: Malignant melanoma; Cell adhesion molecules; L1; Metastasis

1. Introduction

Cutaneous malignant melanoma is rapidly rising in incidence and, worryingly, the prognosis for melanoma patients once it has metastasised is fatal [1]. Therefore, it is important to define those factors which favour metastatic spread in order to develop rational therapeutic strategies. Metastasis formation is a multistep phenomenon involving several different cell-to-cell and cell-to-matrix interactions, in which cell adhesion molecules are known to play a key functional role. Initially, cell adhesion within the primary tumour has to be lost, implying that cell adhesion molecules have to be downregulated and/or mutated to a non-functional state, in order to allow the disaggregation of tumour cells from the primary site. Secondly, contact of the metastatic cells with the endothelial cells at the future metastatic site also requires interaction between cell adhesion molecules and their ligands. Finally, the formation of solid tumour nodules at the distant site involves the upregulation of cell adhesion molecules [2–4]. Both (over)expression and downregulation of cell adhesion molecules have been associated with metastasis in malignant melanoma. Specifically, vascular cell adhesion molecule (VCAM)-1, intracellular adhesion molecule (ICAM)-1 and MUC18 being members of the immunoglobulin superfamily of adhesion molecules have been implicated in the development of metastases in cutaneous malignant melanoma [5–8]. While the loss of melanoma cell adhesion mediated by VCAM-1 has

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0959-8049/02/$ - see front matter
PII: S0959-8049(02)00105-3

been associated with metastatic spread [5], an upregulation of MUC18 and ICAM-1 has been noted in relation to the development of metastases [6,7].

In this contribution, emphasis is placed on the cell adhesion molecule L1, also part of the immunoglobulin superfamily [9]. L1 is a transmembrane protein, which is active in various recognition processes within the nervous system and signal transduction pathways are activated as a consequence of its binding [10,11]. The L1 protein consists of an extracellular region, containing six immunglobulin-like domains followed by five fibronectin III-like repeats, a transmembrane domain and a short, phylogenetically-conserved cytoplasmic domain [12,13]. L1 mediates adhesion both via homophilic and heterophilic mechanisms [13]. Although originally described as a neuronal cell adhesion molecule, L1 expression is not restricted to nervous tissues, but has also been detected in lymphocytes, granulocytes, in epithelial cells of the intestinal and urogenital tract, and in the epidermis [14–17]. In addition to expression in normal tissues, L1 expression has also been detected in several highly malignant tumours, including osteogenic sarcoma, squamous cell carcinoma of the lung, rhabdomyosarcoma and retinoblastoma [18].

In a murine model, it has been shown that the L1-positive human melanoma cell line K1735-M1 disseminated, whereas the L1-negative melanoma cell line K1735–C116 did not disseminate after intravenous (i.v.) injection [19]. However, no data concerning L1 expression in malignant melanoma have been reported in a clinical study. The first aim of this study was to evaluate whether L1 expression is associated with metastasis.

However, L1 expression in human cutaneous malignant melanoma is not only of interest from the point of view of prognostic utility, but is also of interest in order to understand the functional role of L1 in melanoma spread. L1 has recently been characterised as a sialic acid binding lectin, (siglec) with the siglec domain located within the first fibronectin type III repeat, which specifically binds to α2,3 sialic acid residues [20]. To further analyse potential binding partners for the L1 siglec domain, the second aim of this study was to analyse the expression of α2,3 sialic acid residues in the melanoma cells and in the tissue surrounding the tumour by lectin histochemistry using the lectin *Maackia amurensis* agglutinin (MAA), specific for this glycotope.

2. Patients and methods

2.1. Primary cutanous malignant melanoma

Paraffin sections of human cutaneous malignant melanoma from 123 patients, who underwent surgery between 1983 and 1996 in the Dermatological Hospital, University Hospital Eppendorf, Hamburg, Germany, were investigated. The data from 100 patients could be retrieved in the outpatients' clinic and were followed-up for 10 years. Date of diagnosis, surgery, occurrence of first metastasis and death, as well as data of Breslow's tumour thickness and clinical stage were recorded from these original reports. Informed consent of all patients, whose tumours were investigated, had been obtained following institutional guidelines.

2.2. Patient characteristics

The majority of the patients were female (female = 60; male = 40) and the median age was 66.5 years (range 19–97 years). All patients were diagnosed with localised cutaneous melanoma. Forty patients presented with stage IA (tumour thickness <0.75 mm), 23 with stage IB (tumour thickness 0.75–1.5 mm), 32 with stage IIA (tumour thickness 1.6–4 mm) and 5 with stage IIB (tumour thickness >4 mm) tumours; staging was performed according to recommendations of the German Dermatological Society (DDG) [21]. After a follow-up of 10 years, 34 patients showed clinical signs of metastasis and of these, 18 showed haematogenous spread to lung ($n=3$), liver ($n=5$), brain ($n=4$), and skin ($n=6$), and 16 showed lymphatic spread as the primary manifestation of metastasis. During the observation period, 20 patients had died of metastatic disease. Two patients had died because of a disease other than metastatic melanoma.

2.3. Nevocytic nevi

To evaluate L1 expression in benign melanocytes, 12 nevocytic nevi were also analysed for expression of this cellular adhesion molecule (CAM). These comprised three compound nevi, three dermal nevi, three junctional nevi and three dysplastic nevi.

2.4. Metastatic lesions

To analyse L1-expression in metastatic lesions, a second series of melanomas was investigated. Primary melanomas ($n=14$, DDG stages III/IV) and corresponding distant metastases (cutaneous/subcutaneous; $n=9$) and metastasis-positive sentinel lymph nodes (SLN; $n=5$) were obtained from the files of the Fachklinik Hornheide, Universiät Münster, Germany. Histological proof of metastases in the SLNs was confirmed by immunohistochemistry in adjacent sections according to standard procedures as reported elsewhere in Refs. [22,23].

2.5. Evaluation of ulceration and mitotic rate

The presence of ulcerations in the primary melanomas was evaluated microscopically by using haematoxylin

and eosin (H&E)-stained slides. Mitotic rates were determined on H&E-stained slides, as described by Schmoeckel and Braun-Falco in Ref. [24]. The mitotic rate was taken as the mean of the mitotic count in ten high-power fields (magnification ×400).

2.6. Antibody preparation and specificity

Polyclonal antibodies against the extracellular domain of the human L1 protein in fusion with the Fc portion of human IgG1 were produced in rabbits according to established procedures [25]. The human L1-Fc protein was produced in CHO cells as previously described for mouse L1-Fc [26]. The human L1-Fc construct was generated as described and tested for biological activity in neurite outgrowth in cell culture [27]. The antibodies were purified by affinity chromatography using a human immunoglobulin column and tested by Western blot analysis on human brain homogenate yielding two major bands at 200 and 140 kD as similarily described for mouse L1 [25]. Antibodies did not react with the Fc portion of human IgG1 as tested by Western blot and enzyme-linked immunosorbent assay (ELISA) using human NCAM-Fc or mouse MAG-Fc as antigens. In histological sections of adult human cerebellum, the antibodies showed the same characteristic staining pattern as seen for poly- and monoclonal antibodies to mouse L1 in adult mouse cerebellum.

2.7. L1 immunohistochemistry

Deparaffinised, 5 μm thick sections were rehydrated through a series of graded ethanols and rinsed in Tris-buffered saline (TBS pH 7.6) with 0.1% Tween 20 (Sigma, Steinheim, FRG) added. All antibodies were diluted in antibody diluent with background reducing components (DAKO, Glostrup, Denmark). The slides were exposed to 0.015 g protease XXIV (Sigma, Steinheim, FRG) dissolved in 150 ml TBS at 37 °C for 7 min. Afterwards the sections were rinsed three times in TBS +0.1% Tween (4 °C) for 5 min and incubated subsequently with 10% normal swine serum (DAKO, Glostrup, Denmark) for 30 min in a humid chamber at 4 °C. This was followed by an incubation overnight with a 1:100 diluted polyclonal rabbit anti-human L1 antibody. The next morning, sections were first washed three times in TBS for 5 min and then incubated with a 1:200 diluted biotinylated swine anti-rabbit antibody (DAKO, Glostrup, Denmark) for 40 min at room temperature. After careful washes, an incubation with an avidin-alkaline phosphatase complex (ABC kit, Vectastain, Vector, Burlingame, CA) for 30 min followed and thereafter, additional washes in TBS were performed. Alkaline phosphatase activity was visualised using Naphthol-AS-bisphosphate as a substrate and New Fuchsin was used for simultaneous coupling. Slides were counterstained with Mayer's hemalum diluted 1:1 in distilled water for ten seconds, blued under running tap water and mounted with Crystal Mount (Biomeda, Foster City, CA). As an appropriate positive control, a human peripheral nerve was used. Additionally, peripheral nerves within the tissue sections under study served as internal positive controls and for the evaluation of the staining intensity. Central nervous myelin as represented by an optic nerve served as an appropriate negative control.

2.8. Lectin histochemistry

Binding of the lectin MAA was re-evaluated in the same series of melanomas, as already published in Ref. [28]. This time, α2,3 sialic acid residue expression was specifically evaluated in the malignant cells, in the endothelia of the tumour vasculature, and in normal blood vessels surrounding the melanoma. The specifity of MAA binding was controlled by neuraminidase predigestion [29].

2.9. Evaluation of the staining pattern and statistical analysis

The staining of the melanoma cells was recorded by two independent observers, who were unaware of the clinical outcome at the time of evaluation. The 100 melanomas were divided into L1-positive and L1-negative cases. A melanoma was regarded as positive, when at least 20% of the melanoma cells were stained. Additionally, the intensity of positive staining from weak (+) to intense staining (+++) was recorded. The observers agreed with the rating in 98 cases; in the remaining 2 cases, consensus was achieved after discussion. For documentation, the slides were examined under a Zeiss Axioplan photomicroscope (Oberkochem, Germany) using a contrast filter and photographed with a Kodak Ektachrome 64T colour film.

The significance of the association between L1 expression in primary malignant melanoma and patients' disease-free survival was analysed unadjusted for tumour thickness, applying the log-rank test to Kaplan-Meier estimates of survival curves [30] using Graph Pad Prism (Intuitive Software for Science, San Diego, CA) on an IBM compatible microcomputer. Furthermore, an univariate score test for the predictive value of all four variables (L1 expression, ulceration, stage and mitotic rate) and multivariate Cox regression analysis for expression of L1 adjusted for clinical stage, presence of ulceration and mitotic rate (continuous) were performed using the Statistical Package for the Social Sciences (SPSS) for Windows version 10 (SPSS Inc., Chicago, Illinois, USA). The correlation between L1 expression, occurrence of metastases, ulceration and tumour thick-

ness were analysed applying the Pearson correlation test using Graph Pad Prism (Intuitive Software for Science, San Diego, CA).

3. Results

3.1. L1 expression

The optic nerve was non-reactive for the L1-antibody (Fig. 1). Epidermal keratinocytes of the skin adjacent to the melanomas and to the nevocytic nevi showed weak (+) to moderate (++) L1 immunoreactivity. However, no differences in the intensity of L1 immunoreactivity in the keratinocytes adjacent to melanomas or adjacent to the nevocytic nevi was apparent. Intense (+++) L1 immunoreactivity was found in endothelial cells of the blood vessels. Furthermore, peripheral nerves within the connective tissue in all cases showed intense L1 immunoreactivity, which served as appropriate internal positive controls (Fig. 2). L1 reactivity was not identified in the melanocytes of any of the 12 nevocytic nevi (Fig. 3). Within the series of melanomas, L1-immunostaining exhibited an unequivocal staining pattern: either the majority of tumour cell clusters were L1-immunoreactive or no L1 immunoreactivity at all could be detected. Forty-two melanomas showed binding of the anti-L1 antibody and 58 showed no immunoreactivity. The vast majority of the positive melanoma cells showed intense (+++) cell membrane staining, while the cytoplasmic staining intensity ranged from weak (+) to intense (+++) staining (Fig. 4). Twenty-four of these 42 L1-positive melanomas had metastasised, of which 14 showed haematogenous spread to liver ($n=3$), lung ($n=2$), brain ($n=3$) and skin (cutaneous and/or subcutaneous: $n=6$) and 10 had metastasised via the lymphatic route. Of the 10 metastatic/L1-negative patients, 4 showed hematogenous spread and 6 showed lymphatic spread.

The connective tissue adjacent to melanomas and to nevocytic nevi showed no L1 immunoreactivity. No changes in the L1 expression pattern in the connective tissue surrounding the tumour with increasing tumour thickness or tendency to metastasise were apparent.

3.2. Metastatic lesions

In the 14 patients whose metastases were examined for L1 expression, the primary lesions were all L1-positive. The corresponding nine distant metastases showed homogenous moderate (++) L1 expression in all malignant cells (Fig. 5) and the melanoma cells in the five metastatic sentinel lymph nodes also showed moderate (++) L1-immunoreactivity.

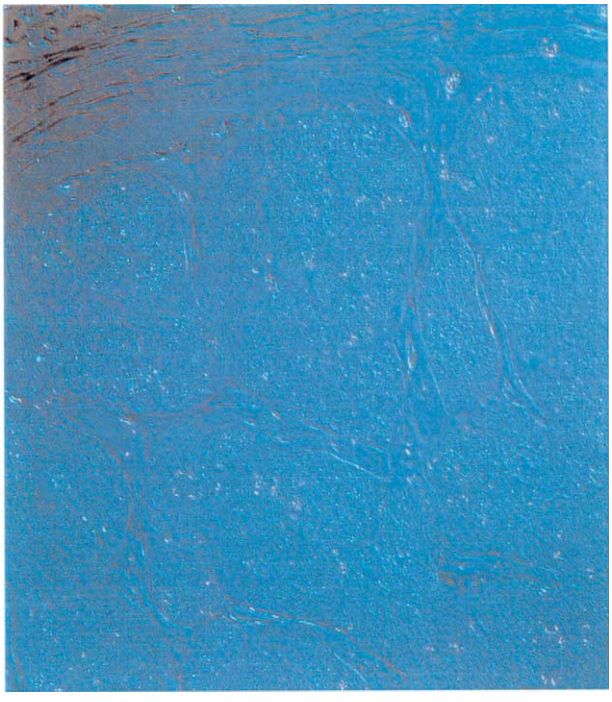

Fig. 1. L1 immunostaining of an optic nerve. The optic nerve representing central myelin was non-immunoreactive to the L1 antibody. Magnification × 70, photographed with differential interference contrast (DIC) technique.

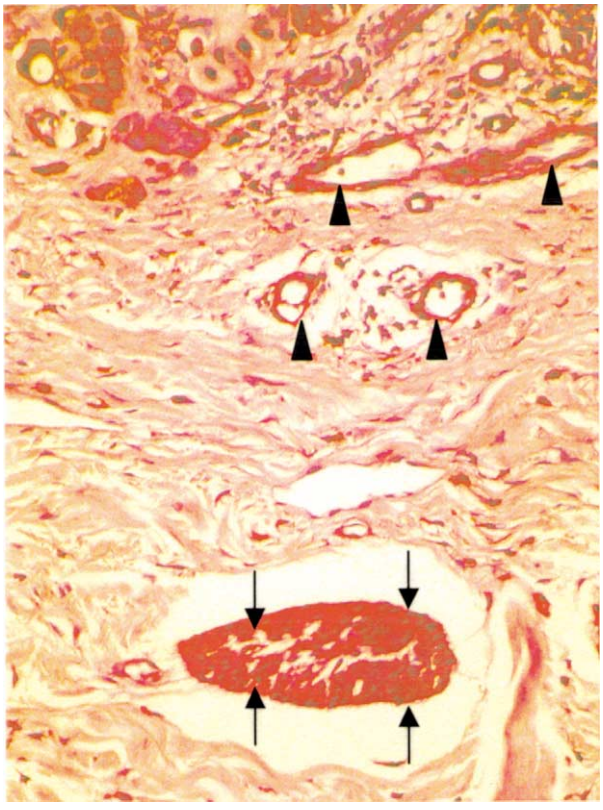

Fig. 2. L1 immunostaining of the surrounding tissues of the melanoma. Peripheral nerves (arrows) showed intense L1-immunoreactivity and served as internal positive controls in each case. Endothelial cells of the blood vessels (arrowheads) were also L1-positive. Magnification ×370.

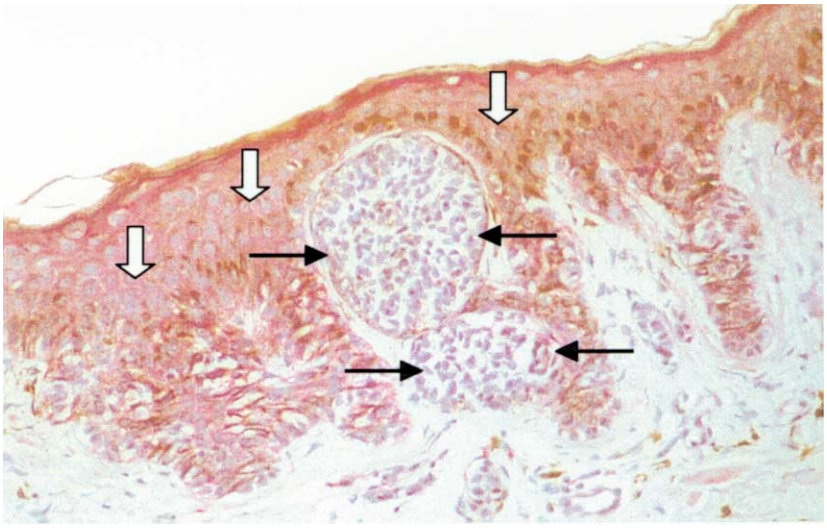

Fig. 3. L1 immunostaining of a compound nevus. Melanocytes within the nevocytic lesion (black arrows) did not show L1-immunoreactivity, whereas the epidermal keratinocytes (white arrows) showed weak to moderate L1 immunoreactivity. Magnification ×185.

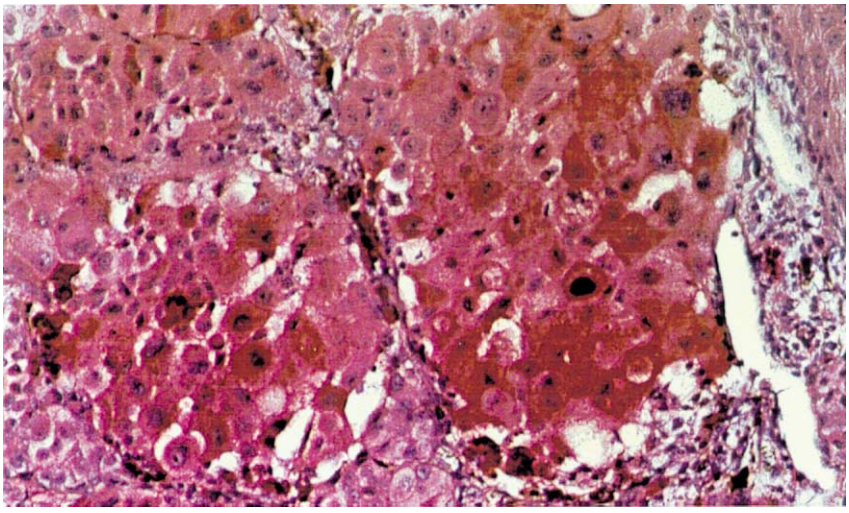

Fig. 4. L1 immunostaining of a malignant melanoma. Note that the majority of tumour cells were stained. Intense (+ + +) staining of the cell membrane as well as weak (+) to intense (+ + +) staining of the metastatic phenotype of malignant melanoma was observed. Magnification ×370.

3.3. Expression of α2,3 sialic acid residues

None of the 100 melanomas under study showed binding of MAA, indicating that no terminal α2,3 sialic acid residues were expressed on the cell membrane of the malignant melanoma. Endothelial cells of the tumour vessels and the normal vasculature in the tumour surrounding tissue were also negative for MAA. However, moderate (+ +) MAA binding was detected in the reticular layer of the connective tissue (Fig. 6) and sebaceous glands exhibited strong (+ + +) binding of this lectin. No changes in MAA binding pattern in the connective tissue surrounding the tumour with increasing tumour thickness or tendency to metastasise were apparent. Neuraminidase pre-digestion did not reduce the intensity of the MAA staining.

3.4. Ulceration and mitotic rate

Ulceration was present in 13 of the 100 melanomas investigated and of these, eight had metastasised. The mean mitotic rate was 1.094 (range 0.0–6.0; standard error of the mean (SEM) 0.133; 95% Confidence Interval (CI) 0.831–1.357). For statistical analyses, the mitotic rate was taken as a continuous variable.

3.5. Statistical analyses

3.5.1. Univariate analysis

The log-rank test of significance between Kaplan-Meier curves for L1-positive versus L1-negative melanomas revealed that patients with L1-positive melanomas proved to have a significantly higher risk of

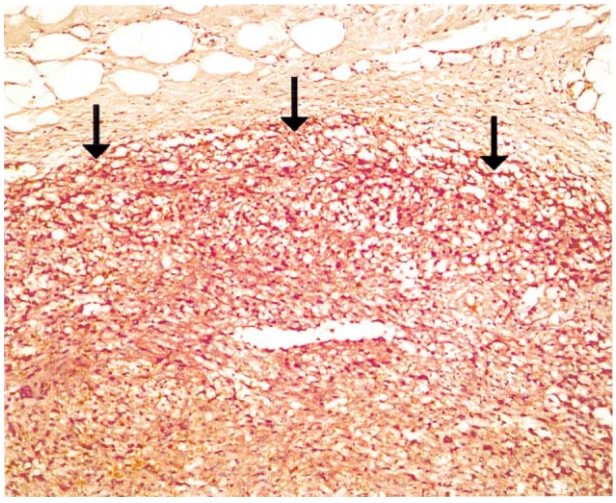

Fig. 5. L1 immunostaining of a distant metastasis. Note that all melanoma cells showed moderate (++) L1 immunoreactivity. Magnification ×185.

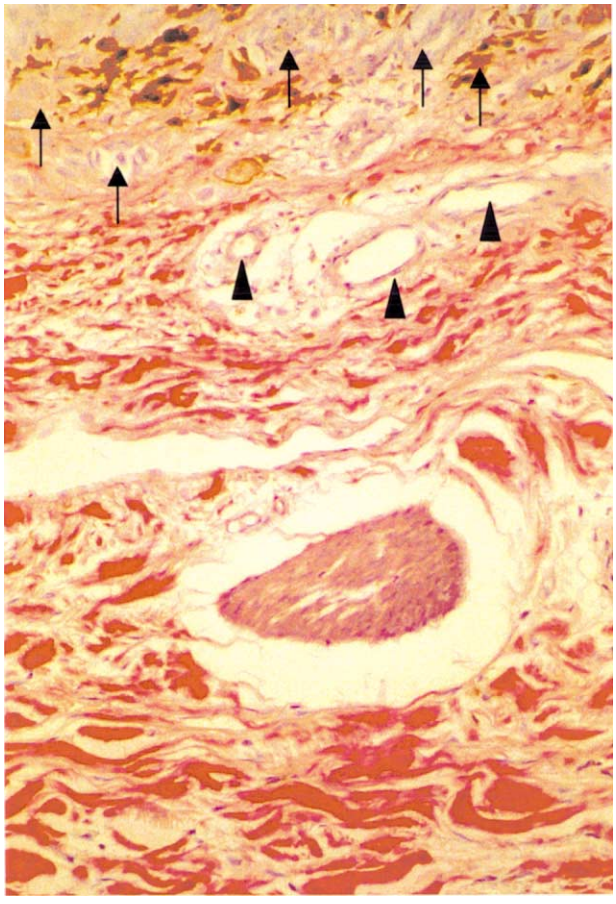

Fig. 6. MAA binding in malignant melanoma and tissues surrounding the tumour. The connective tissue showed moderate (++) MAA binding, whereas melanoma cells (arrows) and endothelial cells of the blood vessels (arrowheads) were MAA-negative. Magnification ×370.

metastasis than those with L1-negative tumours, ($P<0.0001$; Fig. 7). No statistically significant difference between haematogenous versus lymphatic spread for L1-positive melanomas was detected.

3.5.2. Multivariate analysis

At step 0 in a multivariate Cox regression with forward selection of the variables, the score test indicated that L1-expression, presence of ulceration, tumour thickness and mitotic rate were significant predictors for metastasis in univariate analysis (Table 1). However, only L1 expression status ($P<0.0005$) and tumour thickness ($P<0.0005$) were independent predictors of metastasis (Table 2).

Patients, whose melanomas expressed L1, had a 4.384-fold higher risk of metastasis over patients whose melanomas did not express this adhesion molecule (95%CI: 2.082–9.229; Table 2). The mitotic rate and

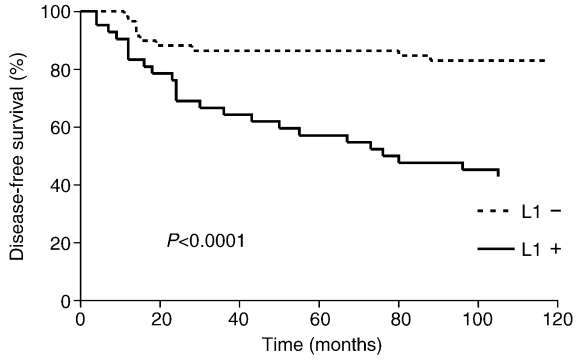

Fig. 7. Disease-free interval in patients, whose primary malignant melanoma were (L1 +) or were not (L1−) immunoreactive with the L1 antibody. Patients with L1-positive melanomas had a significantly higher risk for the development of metastases ($P<0.0001$).

Table 1
Univariate score test for the predictive value of markers for metastasis in 100 DDG stage I and II melanomas

Variable	Score	*P* value
L1	17.026	<0.0005
Ulceration	8.837	0.003
Mitotic rate	8.247	0.004
Tumour thickness (categorial: ⩽1.5 versus >1.5mm)	14.540	<0.0005

Table 2
Estimated hazard ratios and 95% confidence intervals of L1 expression and tumour stage as predictors for metastasis in the multivariate Cox regression model

Variable	Hazards ratio	95% Confidence interval	*P* value
L1-expression	4.384	2.082–9.229	<0.0005
Tumour thickness (categorial: ⩽1.5 versus >1.5 mm)	3.720	1.848–7.488	<0.0005

presence of ulceration lost their predictive significance in the multivariate model (only a non-significant change of −2 log-likelihood of 1.53 was observed with 2 degrees of freedom (d.f.); ($P=0.465$) after forcing the mitotic rate and presence of ulceration in the model with L1 expression and tumour stage).

3.5.3. Correlation between the different predictors for metastasis

The Pearson correlation analysis revealed a significant positive correlation between L1 expression and metastasis ($P<0.0001$) as well as between L1 expression and mitotic rate ($P=0.0001$). L1 expression was not significantly correlated with tumour thickness or the presence of ulceration (Table 3).

4. Discussion

The present study was designed to (i) investigate the possible association between the expression of the cell adhesion molecule L1 in cutaneous malignant melanoma and metastasis formation in a clinical situation and (ii) to compare its expression with the expression of α2,3 sialic acid residues, which can serve as a L1 ligand [14].

The results demonstrate that the expression of L1 in primary cutaneous malignant melanoma is significantly associated with metastatic spread ($P<0.0001$) and multivariate Cox regression analysis confirmed that L1 expression is an independent predictor for the risk of metastasis. The considerable predictive prognostic power of L1 expression is highlighted by incorporating other known prognostic factors including tumour thickness, ulceration of the primary tumour, and mitotic rate into the multivariate analysis [31–33]. Using this tool, only L1-expression and tumour stage had an independent predictive value for outcome in our study. Presence of ulceration and mitotic rate were not significant in the multivariate analysis in connection with L1-expression and tumour stage, while in the univariate analysis all four variables were significant predictors for metastasis. Hence, determination of L1-expression status in cutaneous malignant melanoma improves the accuracy of risk estimations in melanoma patients.

Table 3
Summary of the Pearson correlation analyses between the different predictors for metastasis, 95% confidence intervals and corresponding P values (two tailed)

Variable	Pearson r	95% Confidence interval	P value
L1: metastasis	0.4157	0.2388 – 0.5660	<0.0001
L1: tumour thickness	0.0877	−0.1106 – 0.2794	0.3854
L1: ulceration	0.1530	−0.4479 – 0.3393	0.1285
L1: mitotic rate	0.3766	0.1946 – 0.5336	0.0001

The American Joint Committee on Cancer recommended tumour thickness and ulceration as sole criteria for the T (tumour)-classification in melanoma [32]. However, there is still a clinical need to improve the accuracy of risk estimations obtained with these accepted markers, as there are thin melanomas at time of surgery which are very aggressive, while there are thick melanomas which do not develop metastasis. Thus, a variety of immunohistochemical markers have been identified as adjunct prognostic parameters in primary melanomas. Amongst these, loss of melastatin expression [34], binding of *Peanut* [35] and *Helix pomatia* agglutinin [28] and expression of β3-integrin [36] were particularly valuable as the cohorts included at least 100 patients with follow-up periods of 5–10 years and multivariate analysis including tumour thickness was used to establish the prognostic value of these markers. This study gives evidence that determination of L1 expression has a considerable higher predictive power than tumour thickness or ulceration (Table 1).

Determination of L1 expression status is not only of prognostic interest, but is important from the tumour biological point of view. L1 expression was significantly correlated with metastasis ($P<0.0001$) and mitotic rate ($P=0.0001$). However, L1 was not correlated with ulceration or tumour thickness. The statistical analysis implies that L1 expression is linked to cell proliferation. The biological significance of this statistical finding has to be investigated. As the L1 molecule is absent in normal human melanocytes (none of the nevi expressed L1, Fig. 3) and is only expressed in a few non-metastatic melanomas, the neo-expression of the L1 protein seems to positively influence the metastatic behaviour of melanomas. However, L1 expression did not predict for haematogenous versus lymphatic spread of the metastatic melanoma cells, indicating that joint adhesion mechanisms operate in both metastatic ways. The potential role of L1 in melanoma metastasis is further stressed by the fact that L1 expression was preserved in the metastatic deposits.

Initially, it might seem counterintuitive that cell adhesion molecules are upregulated in metastatic tumours, because the first step of the metastatic cascade is the loosening of the putative metastatic cells from the tumour mass, whereby a downregulation of adhesion molecules would be expected. However, increased cell adhesion, e.g. at the site of the interaction of the metastatic cell with the endothelium in the target organ would be compatible with the upregulation of cell adhesion molecules. Upregulation of a cell adhesion molecule in connection with metastasis therefore implies heterotypic adhesive interactions. Therefore, our data suggest that the underlying adhesive mechanism promoted by L1 in melanoma metastasis in humans would be heterotypic. This is in agreement with observations in M21 melanoma cells *in vitro*, which despite high levels

of L1 expression do not express significant homotypic L1–L1 adhesion, but heterotypic integrin-dependent adhesive interactions [19].

Analysis of MAA binding revealed that the melanoma cells do not express α2,3 sialic acid residues on the cell surface and therefore binding between the melanoma cells via the L1-α2,3 sialic acid can be excluded. This again would suggest that L1 may be instrumental in later steps within the metastatic cascade.

One pivotal step in the process of metastatic spread is the binding of malignant cells to endothelial cells of the vasculature followed by extravasation. Since L1-mediated adhesion of murine leucocytes to endothelial cells has been previously reported in Ref. [37], a similar adhesive process for the melanoma cells to the endothelia in the target organ of metastasis could operate in humans as well. The observation that the human K1735-M1 cell line, which expresses L1, is able to give rise to experimental lung metastasis in immunodeficient mice after i.v. injection, while the L1-negative cell line –C116-does not form metastases [19], is an experimental proof of this hypothesis. In this animal model of melanoma spread, one rate-limiting step for the metastatic capacity of the i.v. injected tumour cells would be the adhesion to the endothelial cells and extravasation as the cells were i.v. injected and it was thus not necessary for them to loosen from the primary site. Our findings would implicate that this mechanism operates in humans as well. To analyse, whether this adhesion of the malignant melanoma cells to the endothelium is promoted by the siglec domain of L1, we additionally evaluated the expression of α2,3 sialic acid residues in the endothelial cells. Since α2,3 sialic acid residues were absent in the vasculature, adhesion of the metastatic cells to the endothelium is not mediated by the siglec function of L1.

MAA-staining revealed that α2–3 sialic acid was expressed in the reticular layer of the connective tissue. However, no reduction of MAA staining intensity was achieved by neuraminidase pre-digestion, suggesting that binding of MAA to the reticular fibres was non-specific. Adhesion via this mechanism, therefore, seems unlikely. In addition to its function as a cell surface adhesion molecule, L1 can be shed from the cell membrane to be deposited in the extracellular matrix (ECM) [38,39]. The shed L1 is functionally intact and supports integrin-mediated cell adhesion and migration [38], suggesting a further potential role for L1 as a matrix constituent as well. By shedding L1, malignant melanoma cells could modify their extracellular environment, which might be important for integrin- or L1-mediated cell spreading and motility. As integrin expression is associated with tumour progression in malignant melanoma [40], a co-operation between L1 and integrins seems likely. Furthermore, the integrin α9β1, which is also present on melanoma cells [41], has been identified as a L1 binding partner [42]. Since α9β1 integrin is able to promote neutrophil transendothelial cell migration via an interaction with VCAM-1 [43], it is conceivable that L1-α9β1 integrin interaction may also modulate transendothelial cell migration in malignant melanoma. Hence, L1 may have a dual function both as a cell adhesion molecule and as a substrate adhesion molecule. However, the potential functional role of L1 in melanoma metastasis cannot be resolved from the clinical data alone and will have to be substantiated in ongoing functional experiments.

In conclusion, this study indicates that determination of L1 expression status in primary cutaneous malignant melanoma improves the accuracy of risk estimations obtained with standard markers.

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