High Affinity Interaction of Integrin α4β1 (VLA-4) and Vascular Cell Adhesion Molecule 1 (VCAM-1) Enhances Migration of Human Melanoma Cells Across Activated Endothelial Cell Layers

MARTIN KLEMKE, TATJANA WESCHENFELDER, MATHIAS H. KONSTANDIN, AND YVONNE SAMSTAG*

Institute for Immunology, University of Heidelberg, Heidelberg, Germany

The capacity of tumor cells to form metastatic foci correlates with their ability to interact with and migrate through endothelial cell layers. This process involves multiple adhesive interactions between tumor cells and the endothelium. Only little is known about the molecular nature of these interactions during extravasation of tumor cells. In human melanoma cells, the integrin αvβ3 is involved in transendothelial migration and its expression correlates with metastasis. However, many human melanoma cells do not express β3 integrins. Therefore, it remained unclear how these cells undergo transendothelial migration. In this study we show that human melanoma cells with different metastatic potency, which do not express β2 or β3 integrins, express the VCAM-1 receptor α4β1. VCAM-1 is up-regulated on activated endothelial cells and is known to promote transendothelial migration of leukocytes. Interestingly, despite comparable cell surface levels of α4β1, only the highly metastatic melanoma cell lines MV3 and BLM, but not the low metastatic cell lines IF6 and 530, bind VCAM-1 with high affinity without further stimulation, and are therefore able to adhere to and migrate on isolated VCAM-1. Moreover, we demonstrate that function-blocking antibodies against the integrin α4β1, as well as siRNA-mediated knock-down of the α4 subunit in these highly metastatic human melanoma cells reduce their transendothelial migration. These data imply that only high affinity interactions between the integrin α4β1 on melanoma cells and VCAM-1 on activated endothelial cells may enhance the metastatic capacity of human β2/β3-negative melanoma cells.

*Correspondence to: Yvonne Samstag, Institute for Immunology, University of Heidelberg, Im Neuenheimer Feld 305, 69120 Heidelberg, Germany. E-mail: yvonne.samstag@urz.uni-heidelberg.de

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cells (Chan and Aruffo, 1993; Masumoto and Hemler, 1993; Alon et al., 1995). These properties result from affinity regulation due to ‘inside-out’ signaling (Shimaoka et al., 2002; Takagi and Springer, 2002). Endothelial cells express the ligand for the integrin α4β1, VCAM-1, on their surface only after inflammatory stimuli, as for example TNF-α (Xu et al., 1994; Haraldsen et al., 1996).

In this study, we demonstrate that only highly metastatic human melanoma cells expressed the α4β1 integrin in its high affinity conformation at the cell surface and adhered to and migrated on VCAM-1. In contrast, human melanoma cells of low metastatic potential expressed the α4β1 integrin in its low affinity conformation. Moreover, transendothelial migration of the highly metastatic melanoma cells was supported through interaction of the integrin α4β1 with its ligand VCAM-1 on the surface of activated endothelial cells. This suggests that highly metastatic melanoma cells preferentially leave the blood vessels at sites of inflammation. Therefore, blocking the α4β1 integrin on metastatic melanoma cells or VCAM-1 on activated endothelial cells may be a valuable approach to interfere with tumor metastasis.

Materials and Methods

Cell culture

The human melanoma cell lines S30, I6F, BLM and MV3 (a generous gift of Dr. van Muijen, University Hospital, Nijmegen, The Netherlands) were grown in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), non essential amino acids, 2 mM glutamine, 25 mM HEPES, 100 U/ml penicillin and 100 mg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO2. HMEC-1 cells (Ades et al., 1992) were grown in MCDB 131 medium (Invitrogen, Karlsruhe, Germany) supplemented with 10% FCS, 1 μg/ml hydrocortisone, 10 ng/ml epidermal growth factor, 100 U/ml penicillin and 100 mg/ml streptomycin at 37°C in a humidified atmosphere containing 5% CO2.

Transfection of melanoma cells with siRNA

The siRNA against the human integrin subunit α4 (ON-TARGETplus SMARTpool L-005189-00-0005, Human iTGA4) as well as non-targeting control siRNA (ON-TARGETplus siCONTROL Non-targeting pool D-001810-10-05) were obtained from Dharmacon. Human melanoma cells were transfected using the Lipofectamine 2000 (LF2000) reagent (Invitrogen) following the manufacturer’s instructions. Briefly, melanoma cells were seeded in a 24-well plate at 1×105 cells per well and transfected with 2.5 μg (100 nM) of the respective siRNA. Melanoma cells were incubated for 48–72 h until they were analyzed for protein knock-down.

Antibodies

The function blocking monoclonal antibody against the integrin subunit α4 and the monoclonal antibody against VCAM-I were from CHEMICON. The monoclonal antibody against the integrin subunit αL and the monoclonal antibody against ICAM-I were from DAKO. The directly labeled monoclonal antibodies against the integrin subunits β1, β2, and β3 were obtained from BD Biosciences (Heidelberg, Germany). All monoclonal antibodies used are of the IgG1 isotype. IgG1 isotype-matched control antibodies were from BD Biosciences. The R-Phycoerythrin-conjugated donkey anti-mouse IgG F(ab')2 fragments and the R-Phycoerythrin-conjugated goat anti-human Fcy fragment specific F(ab')2 fragments were purchased from Jackson Immunoresearch (Baltimore).

Flow cytometry

For cell-surface staining, melanoma cells were released from the cell culture dish by incubation with PBS containing 4 mM EDTA for 10 min at 37°C and washed in 200 μl FACS medium consisting of PBS containing 1 mM CaCl2, 0.5 mM MgCl2, 5% FCS (w/v), 0.5% BSA (w/v) and 0.1% sodium azide. Then 2×105 melanoma cells were incubated for 30 min at 4°C in 50 μl of FACS medium containing the respective monoclonal antibodies at the indicated concentrations. Melanoma cells were washed twice in 200 μl FACS medium and, if the primary antibodies were not directly labeled, incubated for 20 min at 4°C in 50 μl of FACS medium containing 2 μg/ml R-Phycoerythrin-conjugated donkey anti-mouse IgG F(ab')2 fragments (Jackson ImmuNoResearch). After washing the melanoma cells in 200 μl of FACS medium and then in 200 μl of PBS to remove unbound antibodies, the stained melanoma cells were resuspended in 300 μl of PBS and analyzed by a FACSCalibur cytometer using the CellQuest Pro software (Becton Dickinson, Heidelberg, Germany).

Ligand-complex-based adhesion assay

Assays were performed as described (Konstandin et al., 2006) with minor modifications. For ligand-complex-based adhesion assays, 270 μl of 20 μg/ml VCAM-1/Fc in PBS+ (PBS containing 1 mM CaCl2, 0.5 mM MgCl2 and 0.5% BSA) were incubated at 4°C for 30 min with 86.4 μl of R-PE conjugated anti-human Fcy fragment specific IgG F(ab')2 fragments to generate soluble multimeric VCAM-1/Fc F(ab')2-PE complexes. Melanoma cells were washed twice with PBS+. The cell density was adjusted to 4×105 cells per ml in PBS+. For each sample 45 μl (1.8×105 cells) of this melanoma cell suspension were mixed with 5 μl of the soluble multimeric VCAM-1/Fc F(ab')2-PE complexes and incubated at 37°C for the indicated time. Binding was stopped by the addition of 1 ml 37°C warm 4% PFA in PBS+. Melanoma cells were fixed for 5 min at 37°C and transferred into 1 ml of ice cold PBS+ containing 5% FCS and 0.5% BSA. After pelleting the melanoma cells, bound soluble multimeric VCAM-1/Fc F(ab')2-PE complexes were detected by flow cytometry using a FACScalibur and the CellQuest Pro software (Becton Dickinson). The percentages and mean channel fluorescence intensities (MFI) of labeled melanoma cells were determined.

Haptotaxis and chemotaxis assay

Transwell migration inserts (PET membrane, 8 μm pore-size, 6.4 mm diameter, BD Biosciences) were left either untreated or the underside was coated in a total volume of 350 μl with 20 μg/ml of recombinant VCAM-1/Fc. After washing with migration medium (RPMI 1640 medium supplemented with 0.1% (w/v) BSA), filters were placed into the chambers with the coated membrane side facing the lower compartment and 600 μl of migration medium (haptotaxis) or 600 μl of migration medium supplemented with 10% FCS (chemotaxis) were added to the lower compartment. Melanoma cells were labeled for 15 min at 37°C with 5% CO2 with 1 μM CFDA-SE (Molecular Probes, Eugene) in PBS+ . The labeling solution was removed and the melanoma cells were incubated in culture medium for an additional 30 min at 37°C. Afterwards, melanoma cells were detached from the cell culture dish by incubation with PBS containing 4 mM EDTA for 1 min at 37°C, pelleted, washed, and resuspended in migration medium at a concentration of 2.5×104 cells/ml. To start the assay, 0.5×105 melanoma cells (200 μl) were added to the top of the filter. Chambers were subsequently incubated at 37°C/5% CO2 and melanoma cells were allowed to migrate for 3 h. Then filters were removed and all cells from the upper membrane surface were wiped off with a cotton swab. Filters were then washed, fixed and mounted on glass slides. Melanoma cells, which had migrated to the coated underside of the filter were detected by confocal laser scanning microscopy, and cells in four defined optical fields were counted for each filter.

Transendothelial migration assay

HMEC-1 cells were seeded on the upper side of transwell migration inserts (PET membrane, 8 μm pore-size, 6.4 mm diameter, BD Biosciences) and grown until a confluent monolayer was established. The integrity of the monolayer was checked microscopically and by trypan blue exclusion. HMEC-1 cells were left either untreated or stimulated overnight with 10 ng/ml TNF-α. After washing with migration medium (RPMI 1640 medium supplemented with 0.1% (w/v) BSA), filters were placed into the chambers with the coated membrane side facing the lower compartment and 600 μl of migration medium were added to the lower compartment. Melanoma cells were labeled for 15 min at 37°C with 5% CO2 with 1 μM CFDA-SE (Molecular Probes, Eugene) in PBS+. The labeling solution was removed and the melanoma cells were incubated in culture medium for an additional 30 min at 37°C. Afterwards, melanoma cells were detached from the cell culture dish by incubation with PBS containing 4 mM EDTA for 1 min at 37°C, pelleted, washed, and resuspended in migration medium at a concentration of 2.5×104 cells/ml. To start the assay, 0.5×105 melanoma cells (200 μl) were added to the top of the filter. Chambers were subsequently incubated at 37°C/5% CO2 and melanoma cells were allowed to migrate for 3 h. Then filters were removed and all cells from the upper membrane surface were wiped off with a cotton swab. Filters were then washed, fixed and mounted on glass slides. Melanoma cells, which had migrated to the coated underside of the filter were detected by confocal laser scanning microscopy, and cells in four defined optical fields were counted for each filter.
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Results
β2/β3 integrin-negative human melanoma cells with different metastatic capacity express comparable levels of the integrin α4β1 on their cell surface

Expression of β3 integrins is important for melanoma cell migration and metastasis (Albelda et al., 1990a; Hofmann et al., 2000). However, none of the human melanoma cell lines investigated here (530, IF6, MV3, and BLM) expressed β2 or β3 integrins (Fig. 1 and Table I). Nevertheless, two of them (MV3 and BLM) are highly metastatic upon injection into nude mice (Van Muijen et al., 1991a; van Muijen et al., 1991b) and should therefore be able to efficiently undergo transendothelial migration. We found that all β2/β3 integrin negative melanoma cell lines analyzed in this study express the α4β1 integrin with comparable levels on their surface (Fig. 1 and Table I). This integrin recognizes, besides fibronectin, VCAM-1 (Chan and Arufo, 1993), a cell surface protein expressed by activated endothelial cells (Xu et al., 1994; Haraldsen et al., 1996; Lee et al., 2001; Park et al., 2001). Since VCAM-1 is known to be important for transendothelial migration of hematopoetic cells (Oppenheimer-Marks et al., 1991; Chan and Arufo, 1993; Chuluyan and Issekutz, 1993; Hourihan et al., 1993; Weber and Springer, 1998; Ding et al., 2001), we investigated, whether the integrin α4β1 is involved in transendothelial migration of these human melanoma cell lines of different metastatic potential.

Adhesiveness of α4β1 expressing human melanoma cells to VCAM-1 correlates with their metastatic potential

To undergo transendothelial migration, tumor cells first have to stably adhere to the endothelial cells. As the integrin α4β1 is subject to affinity regulation due to “inside-out”-signaling, we investigated, whether the α4β1 expressing melanoma cells differentially adhere to VCAM-1. To this end, a novel assay that we have recently developed (ligand-complex-based adhesion assay, LC-AA; Konstandin et al., 2006) was employed. This method allows to measure differences in integrin affinity and avidity at the single cell level. Melanoma cells were incubated with soluble multimeric VCAM-1/Fc F(ab’)2 complexes and the percentages and mean fluorescence intensities of VCAM-1/Fc-complex-binding cells were determined by flow cytometry (Fig. 2A). Indeed, binding of a given cell line to multimeric VCAM-1 correlated with the metastatic potential of the respective cell line. Thus, melanoma cells of low metastatic potential (IF6 and 530) bound to VCAM-1/Fc-complexes to a

| Table I. Cell surface expression of integrin subunits |
|----------------|-------|------|------|------|
|                | α4    | β1   | β2   | β3   |
| 530            | 2 ± 1 | 39 ± 9 | 147 ± 10 | 2 ± 1 | 2 ± 0 |
| IF6            | 3 ± 1 | 22 ± 1 | 143 ± 23 | 3 ± 1 | 4 ± 1 |
| MV3            | 3 ± 1 | 36 ± 14 | 113 ± 15 | 2 ± 1 | 2 ± 1 |
| BLM            | 3 ± 1 | 34 ± 10 | 184 ± 18 | 3 ± 1 | 3 ± 1 |

*Shown are the mean MFI (mean fluorescence intensity) values ± SD of three independent measurements. Control: secondary antibody only.
The interaction between the integrin α4β1 and VCAM-1 enhances transendothelial migration of human melanoma cells

To investigate, whether the high affinity interaction between the integrin α4β1 on melanoma cells and VCAM-1 on endothelial cells supports transendothelial migration of human melanoma cells, immobilized human dermal microvascular endothelial cells (HMEC-1; Kielbassa et al., 1998; Lidington et al., 1999; Kielbassa-Schnepp et al., 2001; Eum et al., 2004) were used. As shown by flow cytometry, unstimulated HMEC-1 cells expressed integrin β1 and ICAM-1, but not VCAM-1, on their cell surface (Fig. 4A, upper panels). In accordance with data published by others (Xu et al., 1994; Haraldsen et al., 1996), upon treatment with TNF-α for 18 h, HMEC-1 cells also expressed VCAM-1 (Fig. 4A, lower panels). In addition, the expression level of ICAM-1 increased while the level of the integrin β1 subunit remained unchanged.

**Fig. 3.** Haptotactic and chemotactic migration of the highly metastatic α4β1 expressing human melanoma cells is enhanced in the presence of VCAM-1

Adhesion to endothelial cells is the first step in transendothelial migration. Then cells need to migrate on and finally through the endothelial cell layer. For monocytes it is known that the interaction of α4β1 with VCAM-1 facilitates lateral migration on endothelial cells and thereby supports transendothelial migration (Weber and Springer, 1998). We therefore determined, whether the presence of high affinity VCAM-1 would enhance the haptotactic and chemotactic migration of α4β1 expressing melanoma cells. Cell migration was assayed using transwell inserts which were either left uncoated or coated with VCAM-1/Fc before the assay was performed. For haptotaxis experiments, no chemotactrant was added to the lower compartment, whereas for chemotaxis assays, FCS was added to attract the cells. The presence of VCAM-1/Fc significantly increased the haptotactic (Fig. 3A) as well as chemotactic (Fig. 3B) migration of the two metastatic melanoma cell lines MV3 and BLM, which display high affinity α4β1 on their surface. In marked contrast, the low metastatic melanoma cell lines IF6 and 530 showed almost no migration even in the presence of VCAM-1/Fc. Therefore, the migratory behavior on immobilized VCAM-1 correlated with the metastatic potential of the melanoma cell lines.

![Fig. 3.](image-url)
For the analysis of transendothelial migration, HMEC-1 cells were grown to confluency on transwell inserts and VCAM-1 expression was induced by TNF-α as described (Xu et al., 1994; Haraldsen et al., 1996). Melanoma cells were allowed to transmigrate for 5 h across the endothelial cell layer. As shown in Figure 4B, only the highly metastatic melanoma cell lines MV3 and BLM displayed considerable transendothelial migration. Transmigration already occurred through unstimulated, VCAM-1 negative HMEC-1 cells. It could be significantly enhanced by induction of VCAM-1 expression on the surface of the HMEC-1 cells through TNF-α treatment (Fig. 4B). To confirm the role of integrin α4β1/VCAM-1 interaction in this increase in transendothelial migration, melanoma cells were in parallel incubated with a function blocking antibody against the integrin α4 subunit. This treatment indeed reduced transmigration to the level observed with unstimulated HMEC-1 cells. Note that an unrelated antibody against integrin αL had no effect (Fig. 4B). These data demonstrate that the enhanced transmigration upon TNF-α treatment relies solely on the interaction of high affinity α4β1 with VCAM-1.

**siRNA mediated knock-down of the integrin subunit α4 reduces transendothelial migration of α4β1 expressing human melanoma cells**

To confirm the role of the α4β1 integrin in enhanced transendothelial migration across TNF-α activated endothelial cells by an independent experimental approach, we performed an siRNA-mediated knock-down of the integrin subunit α4 on the two highly metastatic melanoma cell lines MV3 and BLM. Treatment with an integrin α4 specific siRNA reduced the cell surface levels of the integrin subunit α4 by around 70% as compared to a non-targeting control siRNA, whereas the cell surface levels of the integrin subunit β1 did not change (Fig. 5A). In accordance with the experiments performed with function blocking antibodies, treatment of MV3 and BLM cells with the α4 specific siRNA reduced transmigration across TNF-α stimulated HMEC-1 cells down to the level observed with unstimulated HMEC-1 cells (Fig. 5B). These data clearly demonstrate that the interaction of α4β1 and VCAM-1 enhances transendothelial migration of human melanoma cells which express the α4β1 integrin its high affinity state.

**Discussion**

In human melanoma, increased expression of the α4β1 integrin correlates with tumor progression and metastasis (Hart et al., 1991; Moretti et al., 1993; Schadendorf et al., 1993; Schadendorf et al., 1995). However, the underlying mechanism has not been investigated. In this study we demonstrate, that the interaction of the integrin α4β1 with the adhesion molecule VCAM-1 enhances transendothelial migration of human melanoma cells across activated endothelial cells. All melanoma cells used in this study are devoid of β2 and β3 integrins, but express the α4β1 integrin at comparable levels. As this integrin is subject to affinity regulation by "inside-out" signaling (Shimaoka et al., 2002; Takagi and Springer, 2002), we investigated whether the melanoma cell lines with different metastatic capacity used in this study would bind soluble VCAM-1 with similar affinities. Interestingly, the two highly metastatic melanoma cell lines MV3 and BLM bound VCAM-1 with much higher affinity than the 530 and IF6 melanoma cell lines which have low metastatic potential. This finding is likely due to constant activation of signaling pathways that lead to affinity upregulation of the α4β1 integrin in the two melanoma cell lines of high metastatic potential. A role for PKC and intracellular calcium was described to be involved in α4β1 dependent adhesion and cell spreading of human melanoma cells (Saini et al., 1997; Seller and Hart, 2000). However, the exact signaling pathways that regulate α4β1 affinity in human melanoma cells remain to be investigated. Haptotactic as well as chemotactic migration on VCAM-1 also correlated strictly with the metastatic potential of the melanoma cells. The two low metastatic melanoma cell lines S30 and IF6 displayed hardly any haptotactic or chemotactic migration even in the presence of VCAM-1. In contrast, the two highly metastatic melanoma cell lines MV3 and BLM displayed considerable haptotactic and chemotactic migration in the presence of VCAM-1. Interestingly, enhanced migration upon presence of VCAM-1 was most prominent in the haptotaxis migration assay. Therefore, especially in the absence of chemotactic signals, the presence of α4β1 in the high affinity conformation may decide whether tumor cells undergo enhanced transendothelial migration across activated VCAM-1 expressing endothelium.

**Transendothelial migration**

Transendothelial migration of the highly metastatic melanoma cell lines MV3 and BLM occurred already through unstimulated, VCAM-1 negative endothelial cells. As the melanoma cells used in this study are devoid of β2-integrins and do therefore not bind to ICAM-1, transmigration through unstimulated endothelial cells is likely mediated via the interaction of other adhesion molecules than VCAM-1 or ICAM-1. Possible
candidates are PECAM-1 or MCAM, which are expressed by unstimulated endothelial cells (Newman et al., 1990; Albelda et al., 1990b; Bardin et al., 1996) as well as by melanoma cells (Luca et al., 1993; Lutzky et al., 2006), and which are known to be involved in the process of leukocyte transmigration (Petri and Bixel, 2006). VCAM-1 is expressed by endothelial cells only upon activation by inflammatory stimuli like TNF-α or interferon-γ (Xu et al., 1994; Haraldsen et al., 1996; Lee et al., 2001; Park et al., 2001). Accordingly, the transendothelial migration of highly metastatic human melanoma cells was enhanced upon treatment of endothelial cells with TNF-α. Treatment of the melanoma cells with an integrin α4β1 (VCAM-1 receptor) function blocking antibody completely inhibited the enhanced transmigration seen after TNF-α treatment of the endothelial cells, while addition of an unrelated antibody had no effect. Similar results were obtained by siRNA-mediated down-regulation of the integrin α4 subunit on the melanoma cells. Interestingly, the VCAM-1-dependent increase in transmigration of melanoma cells was completely blocked by siRNA mediated knock-down of the integrin subunit α4, although expression of the α4 integrin subunit was not completely down-regulated in these cells. Therefore it is likely that a critical level of α4β1 integrin expression is required for efficient transendothelial migration of melanoma cells. This question could be addressed by comparison of subclones derived from a metastatic melanoma cell line which express different levels of the integrin α4β1 in the high affinity conformation.

As endothelial cells express VCAM-1 on their surface only after inflammatory stimuli, as for example TNF-α, it is tempting to speculate, that integrin α4β1 expressing tumor cells might preferentially leave the blood vessels at sites of inflammation, as it is known for leukocytes (Ebn et al., 1996; Worthylake and Burridge, 2001; Alon and Feigelson, 2002; Luscinias et al., 2002). Recent data have indeed shown that inflammation might be a critical component of tumor progression, and that many cancers arise from sites of infection, chronic irritation and inflammation (for a review see Coussens and Werb (2002)). Tumor-activated macrophages are a significant component of inflammatory infiltrates in tumor tissues. Although these cells may kill tumor cells following activation by for example IL-2 and IL-12 (Brigati et al., 2002; Tsung et al., 2002), they also produce cytokines that potentiate tumor progression (Schoppmann et al., 2002). During the development of human malignant melanoma, tumor-infiltrating activated macrophages produce TGF-β, TNF-α, and IL-1α (Toriu et al., 2000). Through induction of VCAM-1 expression on endothelial cells, these cytokines likely enhance tumor cell migration thereby favoring tumor cell dissemination. Data obtained in mouse tumor models support this hypothesis. Thus, pre-treatment of mice with TNF-α or interleukin 1 leads to an increase in pulmonary metastases upon injection of melanoma cells (Okahara et al., 1994; Garofalo et al., 1995; Higashiyama et al., 1996). This enhanced pulmonary metastasis could be inhibited by either blocking the integrin α4β1 with a monoclonal antibody on the melanoma cells before injection into TNF-α or interleukin 1 treated mice (Okahara et al., 1994; Garofalo et al., 1995; Higashiyama et al., 1996), or by administering a monoclonal antibody against VCAM-1 (Higashiyama et al., 1996). An additional important aspect arises from our findings: Cpg-ODN as proinflammatory factors in combination with preactivated tumor-specific T cells have been successfully used for immunotherapy of cancer in a mouse model (Garbi et al., 2004). Cpg-ODN treatment leads to the up-regulation of ICAM-1 and VCAM-1 on endothelial cells, which in-turn leads to the enhanced recruitment of effector T-cells into the tumor tissue and finally to tumor rejection (Garbi et al., 2004). However, it should be kept in mind that such treatment might not be suitable for α4β1 expressing tumors as in this case it might favor the transendothelial migration not only of effector T-cells but also of α4β1 expressing tumor cells thereby promoting tumor metastasis. The data presented in this study also have implications for gene expression profiling of tumor cells using cDNA microarrays. Often cDNA microarrays are used to compare the expression levels of non-tumor and tumor cells (Gottschlich et al., 2006), or between non-metastatic and metastatic tumor cells (Gottschlich et al., 2006), in order to identify genes which are selectively up- or down-regulated in only one type of cell. In this study, we used two melanoma cell lines of high (MV3 and BLM) and two melanoma cell lines of low (S30 and IF6) metastatic potential. All of these melanoma cell lines expressed comparable levels of α4β1. By using microarray analysis, expression of this integrin would, therefore, not be associated with a specific phenotype. However, using a novel assay (ligand-complex-based adhesion assay; Konstandin et al., 2006) which measures affinity plus avidity of the α4β1 integrin to VCAM-1 on the single cell level, we were able to show that, despite similar expression levels, the binding capacity of α4β1 to VCAM-1 is much lower in melanoma cells with low metastatic potential as compared to melanoma cells with high metastatic potential. In conclusion, α4β1 integrin expression on melanoma cells leads to enhanced transendothelial migration across VCAM-1 expressing activated endothelial cells only if this integrin is present in the high affinity/avidity state. These data demonstrate that rather than mere determination of α4β1 integrin expression (Hart et al., 1991; Moretti et al., 1993; Schadendorf et al., 1993; Schadendorf et al., 1995), the measurement of the affinity plus avidity of α4β1 to VCAM-1 might be used as a predictive marker for metastasis. Consequently, blocking the α4β1 integrin on tumor cells and/or blocking of VCAM-1 on endothelial cells may be a valuable approach to interfere with tumor metastasis.

Literature Cited


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