Clinical Significance of αvβ3 Integrin and Intercellular Adhesion Molecule-1 Expression in Cutaneous Malignant Melanoma Lesions

Pier Giorgio Natali, Carl V. Hamby, Brunhilde Felding-Habermann, Bitao Liang, Maria R. Nicotra, Franco Di Filippo, Diana Giannarelli, Massimo Temponi, and Soldano Ferrone

ABSTRACT

Several lines of experimental evidence in in vitro and animal model systems suggest that the integrin αvβ3 plays a role in the tumorigenicity of human melanoma cells and that the blocking of αvβ3 ligand binding can inhibit tumor progression. However, there is only scanty information about the role of αvβ3 in malignant melanoma in a clinical setting. Therefore, in the present study, we have analyzed the distribution in lesions of melanocyte origin and in normal tissues of the αv integrin subunit and of the αvβ3 complex and their association with histopathological and clinical parameters of malignant melanoma. We have used as probes the monoclonal antibodies (mAbs) TP36.1 and VF27.263.15, which we have shown with a combination of serological and immunohistochemical assays to be specific for the αv subunit and for the αvβ3 complex, respectively. In immunohistochemical assays, mAb TP36.1 stained both benign and malignant lesions of melanocyte origin. In contrast, the reactivity of mAb VF27.263.15 was restricted to malignant lesions. Both mAbs displayed differential reactivity with primary melanoma lesions of different histotypes because they stained about 50% of acral lentiginous melanoma and superficial spreading melanoma lesions, at least 80% of nodular melanoma lesions, and none of the uveal melanoma lesions tested. Both mAbs TP36.1 and VF27.263.15 stained about 60% of lymph node metastases and 80% of cutaneous metastases.

Expression of the αvβ3 complex in melanocytic lesions resembles that of intercellular adhesion molecule-1 (ICAM-1) in several respects: (a) both are expressed in a significantly (P < 0.004) larger proportion of malignant than of benign lesions; (b) expression of both molecules in primary melanoma lesions is significantly (P < 0.05) associated with lesion thickness; and (c) expression of both molecules in primary lesions from patients with stage I melanoma is significantly (P < 0.05) associated with lesion thickness. Furthermore, the expression of both markers by cells of the melanocyte lineage dramatically increases with tumor progression in patients (5–12) and with the extent of ICAM-1 release (4). The expression of both markers by cells of the melanocyte lineage dramatically increases with tumor progression in patients (5–12) and with the extent of ICAM-1 release (4).

INTRODUCTION

Human melanoma cells have been found to express a number of adhesion molecules (1). Among them, the αvβ3 integrin and the ICAM-1 have been suggested to play a role in the tumorigenicity of melanoma cells by several experimental and/or clinical findings (1, 2). First, tumorigenicity of human melanoma cell lines in athymic nude mice strongly correlates with αvβ3 integrin expression by malignant cells (3) and with the extent of ICAM release (4). Furthermore, the expression of both markers by cells of the melanocyte lineage dramatically increases with tumor progression in patients (5–12). Last, there is convincing evidence that ICAM-1 expression in primary lesions is correlated positively with their thickness and negatively with disease-free survival in patients with malignant melanoma (6–9).

There is only scanty information about the relationship between the αvβ3 complex expression in primary melanoma lesions and their histopathological characteristics. Immunohistochemical staining of a limited number of primary lesions has surprisingly showed no association between their thickness and αvβ3 complex expression (10). Furthermore, to the best of our knowledge, it is not known whether αvβ3 complex expression in primary melanoma lesions is associated with the clinical course of the disease and whether αvβ3 complex and ICAM-1 expression in primary melanoma lesions complement each other in predicting the prognosis of the disease. Therefore, in the present study, we have addressed these questions with mAbs we have shown to recognize the αvβ3 complex and αv subunit.

MATERIALS AND METHODS

Cell Lines, Purified Receptors, and Tissue Samples. Cultured human melanoma cells Colo 38 and cultured human B-lymphoid cells LG-2 were grown in RPMI 1640 (Life Technologies, Inc., Grand Island, NY) supplemented with 10% FCS (Life Technologies, Inc.) and 2 mM L-glutamine (Life Technologies, Inc.). Cultured human melanoma cells M21 and M211L12 (13) and cultured human lung adenocarcinoma cells UCLA-P3 were grown in DMEM (Life Technologies, Inc.) containing 10% FCS, 20 mM HEPES, and 1 mM pyruvate. The αvβ3 receptor (14) and the αvβ5 receptor (15) proteins were purified from human placenta and platelets, respectively, as described.

Normal tissues and benign and malignant lesions of melanocyte origin were obtained from the surgical pathology section of the Regina Elena Cancer Institute. Tissue samples were snap frozen in liquid nitrogen. Four-μm-thick cryostat sections of each sample were fixed in absolute acetone for 10 min. Fixed sections were either immediately used in immunohistochemical assays or kept frozen at −70°C for at least 6 months with no loss of serological reactivity. Fixed sections were stained with 1% toluidine blue to evaluate the histological features of the lesions. Histological diagnoses were made according to Clark et al. (16), and tumor thickness was evaluated according to Breslow (17).

mAb and Conventional Antisera. mAbs VF27.263.15 and TP36.1, both IgG2b, were obtained from BALB/c mice immunized with SDS-treated melanoma cell membranes and with IFN-γ-treated melanoma cells, respectively.

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2 To whom requests for reprints should be addressed, at Department of Microbiology and Immunology, Basic Science Building, Room 308, New York Medical College, Valhalla, NY 10595. Phone: (914) 993-4175; Fax: (914) 993-4176.

3 The abbreviations used are: ICAM-1, intercellular adhesion molecule-1; mAb, monoclonal antibody; MAA, melanoma associated antigen; ALM, acral lentiginous melanoma; SSM, superficial spreading melanoma; IIF, indirect immunofluorescence; IEP, indirect immunoperoxidase; LMM, lentigo maligna melanoma; NM, nodular melanoma.
Fig. 1. SDS-PAGE analysis of antigens immunoprecipitated from cultured human melanoma cells Colo 38 by mAB VF27.263.15, mAB TP36.1, anti-α6 chain mAB LM142, and anti-αvβ3 complex mAB LM609. NP40 extracts from 125I-labeled (left panel) and 125S)methionine-labeled (right panel) Colo 38 cells were incubated for 16 h at 4°C on a rotator with protein A-Sepharose insolubilized mAB LM142 (Lane A), LM609 (Lane B), VF27.263.15 (Lane C), and TP36.1 (Lane D).

After washing, antigens were eluted from immunoadsorbent beads by boiling with 50 μl of electrophoresis sample buffer and separated by SDS-PAGE under reducing (left panel) or nonreducing (right panel) conditions in 3–15% polyacrylamide gradient gels using the Laemmli buffer system (23). After drying, the gel containing 125I-labeled antigens was autoradiographed using a Kodak XAR-5 film (Eastman Kodak Company, Rochester, NY), and the gel containing 125S-labeled antigens was processed for fluorography as described by Bonner and Laskey (28).

Serological Assays. The ELISA with cells, the binding assay with 125I-labeled mAB, the Scatchard plot analysis of the binding of radiolabeled mAB to cells, and the cross-blocking assay with radiolabeled mAB were performed in 96-well polystyrene microtiter plates (Dynatech Laboratories, Alexandria, VA), as described elsewhere (21, 26). The ELISA with purified receptor proteins was performed using 96-well microtiter plates coated with αvβ3 or αIIbβ3 receptor proteins by an overnight incubation at 4°C with 100 μl of a 1 mg/ml receptor protein solution in NaHCO3, pH 9.5. Plates were blocked with 2% BSA in Tris-buffered saline (10 mM Tris, 150 mM NaCl, pH 7.4) supplemented with 1 mM CaCl2, 1 mM MgCl2, and 1 mM MnCl2. Increasing concentrations of mABs were incubated in plates for 2 h at 4°C. Following three washings, plates were incubated with horseradish peroxidase-conjugated anti-mouse IgG xenoantibodies, washed, and developed with o-phenylenediamine as the substrate. Plates were read in an ELISA reader at 490 nm, and the results were expressed as absorbance values.

IIF staining and flow cytometry of cells was performed as described elsewhere (13). IIF staining of acetone-fixed cryostat tissue sections with mABs (concentrations ranging from 10 to 30 μg/ml) was performed as described (7). IIF staining of tissues with purified mAB (concentrations ranging from 10 to 50 μg/ml) was performed using the ABC Vectastain kit according to the manufacturer’s instructions. Slides were developed using 3-amino-9-ethylcarbazole as the chromogenic substrate and were counterstained with Mayer’s hematoxylin.

as described in detail elsewhere (18). The anti-α6 chain mABs LM142 (2) and AV-8 (13), both IgG1, the anti-β3 chain mAB AV-10, an IgG1 (13), the anti-αvβ3 complex mAB LM609, an IgG1 (2), the anti-αIIbβ3 complex mAB CJ-CP8, an IgG1 (19), the anti-100,000 molecular weight MAAb 376.96, an IgG2a (20), and an anti-ICAM-1 mAB CL203.4, an IgG2a (21), were developed and characterized as described previously.

mAbs were purified from ascitic fluid either by sequential precipitation with caprylic acid and ammonium sulfate (22) or by affinity chromatography on protein A-Sepharose (Pharmacia Biotech, Inc., Uppsala, Sweden). The purity of mAB was analyzed by SDS-PAGE (23). Antibodies were labeled with 125I using the Iodo-Gen method (24). Radiolabeled mAB preparations used in binding assays had at least 80% immunoreactivities, as measured by the method of Lindmo et al. (25).

Rabbit anti-mouse immunoglobulin antibodies were purchased from Jackson Immunoresearch Laboratories (West Grove, PA). FITC-conjugated, anti-mouse IgG (H+L chain) xenoantibodies were purchased from Zymed (San Francisco, CA). FITC-conjugated F(ab')2 fragments of anti-mouse and anti-rabbit immunoglobulin xenoantibodies were purchased from Cappel (Organon Teknika, Turnhout, Belgium). Prior to use, the latter preparations were extensively adsorbed with washed human AB, Rh' RBCs and with insolubilized pooled normal human plasma. The ABC Vectastain kit for immunoperoxidase staining was purchased from Vector Laboratories, Inc. (Burlingame, CA).

Fig. 2. Structural relationship among the antigens recognized by mAB VF27.263.15, mAB TP36.1, anti-αvβ3 chain mAB LM142, and anti-αvβ5 complex mAB LM609. NP40 extracts from 125I-labeled Colo 38 cells were depleted of antigens by five rounds of incubation with protein A-Sepharose insolubilized mABs VF27.263.15, TP36.1, LM142, or LM609. Extracts were then divided into aliquots, and each aliquot was immunoprecipitated separately with mABs VF27.263.15, TP36.1, LM142, or LM609. After washing, antigens were eluted by boiling with 50 μl of electrophoresis buffer and subjected to SDS-PAGE under reducing conditions in 10% polyacrylamide gels. The anti-100,000 molecular weight MAAb 376.96 was used to monitor the specificity of the depletion procedure. After drying, gels were autoradiographed using a Kodak XAR-5 film (Eastman Kodak Company).

IMMUNOPRECIPITATED WITH mAb

A B C D

376.96 VF27.263.15 LM609 TP36.1 LM142

- 160 Kd - 100 Kd

IMMUNODEPLETED WITH mAb

376.96 VF27.263.15 LM609 TP36.1 LM142

1555
96-well microtiter plates coated with av@3i (left panel) or aIIb@3 receptor proteins. mAb TP36.l (- -) and VF27.263.l5 (---) were added to dase-conjugated anti-mouse IgG xenoantibodies and o-phenylenediamine substrate reaction using an ELISA reader at 490 nm. Anti-β3 mAb AV-10 (1 μg/well; C) was used as a positive control.

In immunocytochemistry sections incubated with mouse immunoglobulin instead of the primary mAb served as controls in both IIF and IIP. No difference in sensitivity between IIF and IIP assays was observed; therefore, the assays were used interchangeably. Slides were read by independent readers, and the staining patterns were classified as described in the figure legends.

**Immunoochemical Assays.** Radiolabeling of cells with 125I using the lactoperoxidase method, metabolic labeling with [35S]methionine, solubilization with NP-40, and indirect immunoprecipitation with mAb using protein A-Sepharose CL-4B coated with rabbit anti-mouse immunoglobulin antibodies were performed as described previously (27). One dimensional SDS-PAGE was performed under reducing and nonreducing conditions in 3–15% polyacrylamide gradient gels using the Laemmli buffer system (23). Gels contain 25I-labeled samples were processed for autoradiography using Kodak XAR-5 films (Eastman Kodak Company, Rochester, NY). Gels containing [35S]methionine-labeled samples were fluorographed as described by Bonner and Laskey (28).

**Statistical Analysis.** Fisher's Exact test was used to analyze differences in expression of αv chain and αβ3 complex in benign melanocyte lesions and acrylamide gradient gels using the Log-Rank test to Kaplan-Meier estimates of survival curves.

**RESULTS**

**Specificity of mAbs VF27.263.15 and TP36.1.** Both mAbs VF27.263.15 and TP36.1 reacted in ELISA with cultured human melanoma cells Colo 38 and did not react with cultured human B-lymphoid cells LG-2. The association constants of mAbs VF27.263.15 and TP36.1 for Colo 38 cells are 1.8 × 10^-9 M^-1 and 3.3 × 10^-9 M^-1, respectively.

The SDS-PAGE profiles under reducing and nonreducing conditions of antigens immunoprecipitated by mAbs VF27.263.15 and TP36.1 from radiolabeled cultured melanoma cells Colo 38 resemble those of antigens immunoprecipitated by anti-αvβ3 complex mAb LM609 and by anti-αv chain mAb LM142 (Fig. 1). Immunodepletion experiments defined the structural relationship among the antigenic populations recognized by the four mAbs. As shown in Fig. 2, mAbs VF27.263.15 and LM609 did not immunoprecipitate any component from Colo 38 melanoma cell extracts that had been immunodepleted with either mAb TP36.1 or with mAb LM142. The latter two mAbs, which immunodepleted each other, immunoprecipitated a subpopulation of molecules from the Colo 38 cell extracts that had been immunodepleted with mAbs VF27.263.15 or LM609. The specificity of the immunodepletion was monitored with the anti-100,000 molecular weight MAA mAb 376.96, which immunoprecipitated a 100,000 molecular weight antigen from Colo 38 melanoma cell extracts that had been immunodepleted with any of the four mAbs. mAb 376.96 failed to immunodeplete the antigens immunoprecipitated by the above four mAbs. These results suggest that mAb TP36.1 reacts with the αv subunit and that mAb VF27.263.15 recognizes a determinant expressed by the β3 chain or by the αvβ3 complex, because Colo 38 cells are likely to express more than one αv integrin. αIIbβ3 is not involved in the reactivity of mAb VF27.263.15 with Colo 38 cells extracts, because these cells were not stained in IIF by the mAb LJ-CP8. The latter mAb stained αIIbβ3-transfected M21-LIIb human melanoma cells (13) and human platelets (results not shown). The conclusions about the specificity of mAbs TP36.1 and V27.263.15 were corroborated by the following findings. mAb TP36.1 reacted with melanoma cells M21, which express both αvβ3 and αvβ2 complexes (2), with lung adenocarcinoma cells UCLA-P3, which express αvβ3 but no β3 integrins (29), and with purified αβ2 complex. In contrast, mAb TP36.1 did not react with melanoma cells M21-LIIb, which express αIIbβ2 complex, but do not express αv integrins (13), and with purified αIIbβ3 complex (Fig. 3 and 4). mAb

![Fig. 4. Differential IIF staining by mAbs TP36.1 and VF27.263.15 of cultured human melanoma cell lines with different integrin expression.](image)

**Table 1** Mapping of the determinants recognized by mAbs VF27.263.15, TP36.1, LM142, and LM609 on the cell surface of cultured human melanoma cells Colo 38.

<table>
<thead>
<tr>
<th>Unlabeled mAb</th>
<th>Specificity</th>
<th>VF27.263.15</th>
<th>TP36.1</th>
<th>LM142</th>
<th>LM609</th>
</tr>
</thead>
<tbody>
<tr>
<td>VF27.263.15</td>
<td>αvβ3</td>
<td>97</td>
<td>0</td>
<td>7</td>
<td>99</td>
</tr>
<tr>
<td>TP36.1</td>
<td>αv</td>
<td>14</td>
<td>95</td>
<td>85</td>
<td>0</td>
</tr>
<tr>
<td>LM142</td>
<td>αvβ3</td>
<td>31</td>
<td>84</td>
<td>86</td>
<td>2</td>
</tr>
<tr>
<td>LM609</td>
<td>αvβ3</td>
<td>96</td>
<td>0</td>
<td>15</td>
<td>85</td>
</tr>
</tbody>
</table>

*Colo 38 cells (2 × 10^5 cells/well) were incubated with unlabeled mAb (10 μg/well) in a total volume of 100 μl of PBS-BSA. After a 1-h incubation at 4°C on a rotator, 125I-labeled mAb (2 × 10^5 cpm/well) was added to each well, and the incubation was prolonged for an additional 2 h at 4°C. Plates were then centrifuged, and cell-bound radioactivity was measured in a gamma counter. Results are expressed as percentage of inhibition compared to binding performed in the presence of mouse IgG.*
VF27.263.15 reacted only with M21 melanoma cells and with purified ανβ3 complex (Figs. 3 and 4). The results of cross-blocking experiments suggest that mAb TP36.1 recognizes a determinant that is expressed on the cell surface in close proximity, if it is not identical to that recognized by mAb LM142, because the two mAbs competed for binding to Colo 38 cells (Table 1). The determinant(s) recognized by mAbs TP36.1 and LM142 is distinct and spatially distant from that defined by mAbs VF27.263.15 and LM609, because these mAbs failed to interfere with the binding of mAbs TP36.1 and LM142 to Colo 38 cells. mAbs VF27.263.15 and LM609 recognize spatially close, if not identical, determinants because they inhibit each other's binding to target cells (Table 1).

Expression of αν Chain and ανβ3 Complex in Benign and Malignant Lesions of Melanocyte Origin. Fig. 5 shows representative staining patterns by mAbs VF27.263.15 and TP36.1 of benign and malignant lesions of melanocyte origin. The results are summarized in Table 2 based on immunohistochemical evaluation of a representative panel of surgical biopsies. It is readily apparent that the reactivity pattern of mAb VF27.263.15 was associated with malignant transformation of melanocytes, whereas that of mAb TP36.1 was not. Over a wide range of mAb concentrations (5—50 μg/ml), mAb VF27.263.15 stained none of the intradermal nevi tested, whereas mAb TP36.1 stained 87% of them, a difference that was highly significant (P < 0.004). In contrast, the reactivity of the two mAbs with a panel of primary and metastatic melanoma lesions was concordant in 86% of the cases. Both mAbs stained approximately 45% of primary and 65% of metastatic melanoma lesions. The difference in the proportion of primary and metastatic lesions stained by the two mAbs was not significant (P > 0.09). The two mAbs displayed differential reactivity with primary melanoma lesions of different histotype. Both mAbs stained about 50% of ALM and SSM lesions and at least 80% of NM lesions. Neither mAb stained any of the uveal melanoma lesions tested. Within the group of metastatic lesions, both mAbs stained a higher percentage of cutaneous than of lymph node metastases. However, the difference did not achieve statistical significance (P > 0.18). The reactivity patterns of the two mAbs with autologous metastases are concordant. A summary of the results is shown in Table 3, which includes for comparison purposes the staining by anti-ICAM-1 mAb CL203.4. A high degree of concordance was found in the expression of ανβ3 complex, ICAM-1, and LM609 in the set of metastases analyzed.

### Table 2: Expression of integrin αν chain and ανβ3 complex in benign and malignant lesions of melanocyte origin

<table>
<thead>
<tr>
<th>Lesions</th>
<th>αν chain</th>
<th>ανβ3 complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benign</td>
<td>13/16</td>
<td>0/15</td>
</tr>
<tr>
<td>Blue nevi</td>
<td>0/4</td>
<td>0/4</td>
</tr>
<tr>
<td>Malignant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALM</td>
<td>2/4</td>
<td>2/4</td>
</tr>
<tr>
<td>LMM</td>
<td>1/1</td>
<td>0/1</td>
</tr>
<tr>
<td>NM</td>
<td>5/5</td>
<td>4/5</td>
</tr>
<tr>
<td>SSM</td>
<td>11/21</td>
<td>11/21</td>
</tr>
<tr>
<td>UMb</td>
<td>0/8</td>
<td>0/8</td>
</tr>
<tr>
<td>Metastatic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lymph node</td>
<td>14/24</td>
<td>14/24</td>
</tr>
<tr>
<td>Cutaneous</td>
<td>8/10</td>
<td>8/10</td>
</tr>
</tbody>
</table>

*ανβ3 complex* reactions with anti-αν chain mAb TP36.1 and anti-ανβ3 complex mAb VF27.263.15. Results are indicated as number of positive lesions/number of tested lesions. a UM, uveal melanoma.

Fig. 5. Immunoperoxidase staining with anti-αν chain mAb TP36.1 and with anti-ανβ3 complex mAb VF27.263.15 of frozen sections of surgically removed benign and malignant lesions of melanocyte origin. Cryostat sections of surgically removed intradermal nevi (A and B) and primary (C and D) and metastatic (E and F) melanoma lesions were stained with anti-ανβ3 chain mAb TP36.1 (A, C, and E) or anti-ανβ3 complex mAb VF27.263.15 (B, D, and F) in an avidin-biotin complex immunoperoxidase reaction. mAb TP36.1 (A) stains basal keratinocytes (thin arrows) and nevic cells (large arrows), whereas mAb VF27.263.15 (B) stains neither cell type. The two mAbs stain primary melanoma lesions with variable intensity (C and D) but display a strong and homogeneous staining of metastatic lesions (E and F). Slides were developed with aminoethylcarbazole as the chromogenic substrate and were counterstained with Mayer's hematoxylin. A and B, x 120; C and D, x200; E and F, x300.
ICAM-1 in primary tumors was examined in 20 patients with stage I melanoma who underwent excisional surgery and had at least 7 years of follow-up. Kaplan-Meier estimates of the survival curve for each patient group were tested for significant differences using the Log Rank test. Patients bearing αVβ3-positive tumors had a significantly (P = 0.024) greater probability of disease recurrence within 7 years (Fig. 6A). ICAM-1 expression was also associated with a significantly (P = 0.040) greater probability of disease recurrence (Fig. 6B). The association with clinical outcome became even more pronounced, when the disease-free survival of the patients whose primary lesions were not stained by anti-αVβ3 complex mAb VF27.263.15 but were stained by both mAbs or by neither was compared. The probability of recurrent disease was significantly (P = 0.001) greater for patients whose primary lesions were stained by both mAbs than for those whose lesions were stained by neither mAb (Fig. 6C). Notably, all patients whose primary lesions lacked both αVβ3 and ICAM-1 expression were disease-free 7 years following surgery.

### DISCUSSION

Immunohistochemical staining of surgically removed benign and malignant lesions of melanocyte origin with mAbs TP36.1 and...
VF27.263.15, which we have shown to be specific for the αv chain
and αvβ3 complex, respectively, has corroborated some of the data
published previously and has provided novel information. The αv chain
and the αvβ3 complex are highly concordant in their expression
in primary and metastatic melanoma lesions but display a differential
expression in benign and malignant lesions. In agreement with
the information in the literature (5, 10–12), we have found that the αvβ3
complex is a marker of malignant transformation of melanocytes,
because it is not detectable in benign lesions but is expressed in at
least 50% of primary cutaneous melanoma lesions and in at least 60%
of metastatic lesions. In contrast, the αv chain is expressed in at least
50% of both benign and malignant lesions, suggesting that αv sub-
units associate with β subunits other than β3 in benign lesions of
melanocyte origin. Both the αv chain and the αvβ3 complex are
differentially expressed in primary lesions of different histotype. In
agreement with Ten Berge et al. (31), we have found that neither
molecule is expressed in uveal melanoma lesions. The biological
significance of the latter finding remains to be determined.

The distribution of the αvβ3 complex in lesions of melanocyte
origin resembles that of ICAM-1 in some characteristics but also
displays distinct features. Like ICAM-1 (6–9), αvβ3 has a significantly
higher expression in malignant than in benign lesions. Further-
more, the expression of both markers in primary melanoma lesions
is associated with their thickness. However, the expression of ICAM-1
by melanoma cells is modulated by cytokines such as IFN-γ, inter-
leukin 1, and tumor necrosis factor-α (32, 33), whereas that of the
αvβ3 complex is not (18). The latter difference may have a bearing on
the mechanism(s) underlying the association of the expression of the
αvβ3 complex and ICAM-1 with the thickness of primary melanoma
lesions. The association for ICAM-1 may reflect the secretion of
cytokines by T cells infiltrating the melanoma lesions, whereas the
association for the αvβ3 complex may reflect an intrinsic character-
istic of malignantly transformed melanocytes. Furthermore, ICAM-1
has a significantly higher expression in metastases than in primary
lesions (6–9), whereas the αvβ3 complex has a similar distribution in
both types of lesions. The latter finding parallels that of Danen et al.
(12), who reported a lack of association between αvβ3 complex
expression by melanoma cell lines and their metastatic potential in
nude mice.

Experiments in animal model systems have demonstrated the role
of αvβ3 in tumorigenicity and dissemination of melanoma cells to
lymph nodes (5, 10–12, 34). Our data in a clinical setting do not argue
in favor of lymph node dissemination but strongly suggest that αvβ3
expression plays a role in the aggressive phenotype of melanoma
cells. We have demonstrated for the first time a significant association
between αvβ3 expression in primary melanoma lesions and disease
recurrence in long-term follow-up of patients with stage I disease who
had undergone surgical excision of their primary lesions. ICAM-1
expression was also associated with poor prognosis, consistent with
our findings from a previous study (7). Several mechanisms derived
from animal model systems can account for these findings. Nip et al.
(34) have shown that the αvβ3 complex mediates the attachment and
migration of tumor cells over extracellular matrix proteins, and more
recently, Brooks et al. (35) have reported a direct interaction of the
matrix-degrading metalloproteinase MMP-2 with the αvβ3 complex
on the surface of melanoma cells. The latter association led to en-
hanced levels of proteolytically active MMP-2 and facilitated tumor

Table 5  Distribution of integrin αv chain and αvβ3 complex in normal adult human
tissues of nonlymphoid origin

<table>
<thead>
<tr>
<th>Tissue</th>
<th>αv chain</th>
<th>αvβ3 complex</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epidermal keratinocytes</td>
<td>Basal</td>
<td>—</td>
</tr>
<tr>
<td>Brain</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Parotid</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ductal epithelium</td>
<td>Basolateral</td>
<td>Basolateral</td>
</tr>
<tr>
<td>Acinar epithelium</td>
<td>Basal</td>
<td>—</td>
</tr>
<tr>
<td>Thyroid thyrocytes</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mammary epithelium</td>
<td>var</td>
<td>—</td>
</tr>
<tr>
<td>Lung</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Alveoli</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Bronchi</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Bronchial glands</td>
<td>Basal</td>
<td>—</td>
</tr>
<tr>
<td>Esophagus</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Stomach</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Basal glands</td>
<td>Basal/cytoplasmic</td>
<td>Basal/cytoplasmic</td>
</tr>
<tr>
<td>Surface epithelium</td>
<td>Basolateral</td>
<td>Basolateral</td>
</tr>
<tr>
<td>Colon-rectum</td>
<td>var</td>
<td>—</td>
</tr>
<tr>
<td>Liver biliary ducts</td>
<td>+</td>
<td>NTb</td>
</tr>
<tr>
<td>Pancreatic ductal epithelium</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Kidney</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Proximal tubules</td>
<td>Basal</td>
<td>Basal</td>
</tr>
<tr>
<td>Distal tubules</td>
<td>Basal/cytoplasmic</td>
<td>Basal/cytoplasmic</td>
</tr>
<tr>
<td>Glomeruli</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Bowman’s capsule</td>
<td>+</td>
<td>—</td>
</tr>
<tr>
<td>Urinary bladder</td>
<td>Basal</td>
<td>—</td>
</tr>
<tr>
<td>Prostate</td>
<td>Basal</td>
<td>—</td>
</tr>
<tr>
<td>Testis</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Ovary</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Endometrial epithelium</td>
<td>Basolateral</td>
<td>Basolateral</td>
</tr>
<tr>
<td>Tubal epithelium</td>
<td>Basolateral</td>
<td>Basolateral</td>
</tr>
</tbody>
</table>

a  Surgically removed normal tissues were stained in indirect immunoperoxidase reac-
tions with anti-αv chain mAb TP36.1 and with anti-αvβ3 complex mAb VF27.263.15. The
staining patterns were classified as: —, when no staining was detected; Basal, when the
staining was restricted to basal membranes; Basolateral, when both basal and lateral cell
membranes were stained; cytoplasmic, when cytoplasm was stained; var, when the
staining intensity was variable; and +, when membrane and cytoplasm were stained.

b  NT, not tested.
cell degradation of collagen matrix. Tumor cell proliferation may also be influenced by the αβ complex because it has been shown to bind with insulin receptor substrate-1 and may synergize with growth factors in stimulating cell proliferation (36). Finally, the αβ complex has been shown to protect melanoma cells against apoptotic cell death in vitro (37). These mechanisms are distinct from those suggested for the association of ICAM-1 with a poor prognosis. ICAM-1 can possibly mediate tumor cell invasion by promoting aggregate formation of neutrophils and tumor cells in the circulation with subsequent lodging and invasion into tissue (38), and/or it may have an immunoprotective effect due to shedding from tumor cells, thereby preventing effective contact and destruction by cytotoxic T cells (39, 40). The different roles of αβ and ICAM-1 in the tubules in kidney suggest that renal function will be a critical clinical parameter to monitor in therapies of malignant diseases relying on systemic administration of anti-αβ mAb.

In view of the suggested application of anti-αβ mAb for immunotherapy of malignant diseases (30), the distribution of this molecule in normal tissues deserves some comment: (a) the αβ complex has a more restricted distribution in normal tissues than the α chain; and (b) the expression of the αβ complex on glomeruli and on distal tubules in kidney suggest that renal function will be a critical clinical parameter to monitor in therapies of malignant diseases relying on systemic administration of anti-αβ mAb.

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REFERENCES

Clinical Significance of $\alpha_v\beta_3$ Integrin and Intercellular Adhesion Molecule-1 Expression in Cutaneous Malignant Melanoma Lesions

Pier Giorgio Natali, Carl V. Hamby, Brunhilde Felding-Habermann, et al.


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