Proc. Natl. Acad. Sci. USA
Vol. 91, pp. 8856–8860, September 1994
Cell Biology

Integrin $\alpha_v\beta_3$ rescues melanoma cells from apoptosis in three-dimensional dermal collagen

ANTHONY M. P. MONTGOMERY, RALPH A. REISFELD, AND DAVID A. CHERESH

Department of Immunology, The Scripps Research Institute, La Jolla, CA 92037

Communicated by M. Frederick Hawthorne, May 24, 1994

ABSTRACT **Human melanoma cells required ligation of the integrin $\alpha_v\beta_3$ to sustain viability and growth in three-dimensional dermal collagen. Variant melanoma cells, lacking the α_v subunit, progressed rapidly to apoptosis within this matrix, whereas transfection of these cells with an α_v cDNA restored $\alpha_v\beta_3$ expression and prevented apoptosis. Furthermore, inhibition of $\alpha_v\beta_3$ ligation with a monoclonal antibody promoted cell death. Apoptosis of $\alpha_v(-)$ cells within this matrix could be overcome by the addition of insulin or serum. However, $\alpha_v(+)$ melanoma cells had a significant growth advantage in the presence of these growth factors. Initial adhesion of the melanoma cells to type I collagen depended on ligation of $\alpha_2\beta_1$, but these cells can degrade this collagen to expose cryptic $\alpha_v\beta_3$ binding sites. These findings provide evidence that the survival and growth of transformed cells may be regulated by collagen degradation and integrin-dependent anchorage to this proteolysed matrix.**

The extracellular matrix (ECM) is a dynamic and complex source of molecular information that profoundly regulates cell behavior. Type I collagen, a ubiquitous component of dermal ECM, has been shown to regulate cell adhesion, morphology, migration, viability, growth, and differentiation (1–4). Integrins expressed at the cell surface may convey information provided by dermal collagens across the plasma membrane (5). Three members of the β_1 integrin subfamily ($\alpha_1\beta_1$, $\alpha_2\beta_1$, and $\alpha_3\beta_1$) have been implicated in direct ligation to native type I collagen (4, 6, 7). Binding by both $\alpha_1\beta_1$ and $\alpha_2\beta_1$ depends upon recognition of the tetrapeptide sequence Asp-Gly-Glu-Ala and requires collagen to be in a native helical configuration (4, 6). Heat denaturation of type I collagen disrupts binding by these integrins, while exposing cryptic Arg-Gly-Asp (RGD) adhesive sites that can be ligated by $\alpha_v\beta_3$ (8). Type I collagen can also be denatured at physiological temperatures after proteolysis (9). Together these findings suggest that $\alpha_v\beta_3$ may directly ligate dermal collagen that has been altered during the course of tissue remodeling or infiltration.

Expression of $\alpha_v\beta_3$ has been directly linked to neoplastic progression and tumorigenicity in malignant melanoma (10–12). Preferential expression of $\alpha_v\beta_3$ on vertically invasive primary melanoma cells and within metastatic foci has been documented (11). We have reported (12) that melanoma variants lacking $\alpha_v\beta_3$ are less tumorigenic in the skin of nude mice than their $\alpha_v\beta_3(+)$ counterparts and that restoration of $\alpha_v\beta_3$ expression in these deficient variants restores their tumorigenic potential.

Recently tumor progression has been proposed to be not only a function of cell proliferation but also a product of aberrant cell survival resulting from inappropriate suppression of apoptosis (13). Here we present evidence that tumor cells can suppress apoptosis through a specific integrin–matrix interaction. Thus, we demonstrate that ligation of $\alpha_v\beta_3$ within a three-dimensional dermal collagen matrix prevents apoptosis and promotes melanoma cell growth. Apart from the potential role of $\alpha_v\beta_3$ in normal developmental processes, we propose that this mechanism may be fundamental to the association between $\alpha_v\beta_3$ expression and melanoma tumorigenesis. We further postulate that tumor cells can proteolyse occluding dermal collagen to promote *de novo* ligation of $\alpha_v\beta_3$ and will, in this fashion, regulate their own survival and growth.

MATERIALS AND METHODS

Cells and Cell Culture. Human melanoma cell line M21 was derived in our laboratory from the cell line UCLA-SO-M21, which was provided by D. L. Morton (University of California, Los Angeles). Variant α_v-deficient cells (M21-L) were negatively selected from M21 cells by fluorescence-activated cell sorting (FACS) with anti-α_v monoclonal antibody (mAb) LM142 (14). M21-L cells were subsequently transfected with an α_v-cDNA (12). M21-L4 cells (α_v-reconstituted) and M21-L12 cells (α_v-deficient transfection control) were selected from a panel of transfected subpopulations by FACS with mAb LM142. Cells were maintained in RPMI 1640 medium with either 1% Nutridoma-SP (Boehringer Mannheim) or 10% fetal calf serum (FCS). Nutridoma-SP is a serum-free, chemically defined growth medium that contains insulin as the only growth factor.

Reagents. Integrin-specific mAbs used include LM609 (anti-$\alpha_v\beta_3$; ref. 14), P4C10 (anti-β_1; ref. 15), P3G2 (anti-$\alpha_v\beta_5$; ref. 16), and 6F1 (anti-$\alpha_2\beta_1$; ref. 17). Antibodies P3G2 and P4C10 were provided by E. A. Wayner (University of Minnesota), whereas antibody 6F1 was from B. Coller (State University of New York). Bovine dermal collagen (Vitrogen 100) and rat tail type I collagen were from Celtrix Laboratories (Palo Alto, CA) and Upstate Biotechnology, respectively. Recombinant tissue inhibitor of metalloproteinases 2 (TIMP-2) was provided by Y. A. De Clerck, Children's Hospital of Los Angeles (18).

Preparation of Collagen Gels. Collagen gels were prepared with pepsin-solubilized bovine dermal collagen dissolved in 0.012 M HCl (Vitrogen 100). Vitrogen 100 is 99.9% pure collagen containing 95–98% type I collagen with the remainder being type III (Celtrix Laboratories). Collagen gels were made according to the manufacturer's recommendations. Isotonic collagen solutions were prepared with 10× RPMI 1640 medium and neutralized to pH 7.4 with 0.1 M NaOH. Melanoma cells were resuspended in cold collagen solutions before gelation at 37°C.

Assessment of Cell Morphology. Melanoma cells were seeded into 48-well tissue culture plates at 1×10^5 cells per well and were suspended in 400-μl aliquots of dermal collagen supplemented with 2% FCS as required. Spreading of melanoma cells within collagen gels was assessed at 24-hr intervals with an Olympus CK-2 inverted microscope (Olympus, Tokyo). Cell morphology was determined in three

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Abbreviations: ECM, extracellular matrix; TIMP-2, tissue inhibitor of the metalloproteinases 2; mAb, monoclonal antibody; FCS, fetal calf serum.

randomly selected high-powered fields per well with a minimum of 50 cells observed in each field. Cells were scored as either round or spread, and the results are expressed as average percentage cell spread per high-powered field.

Adhesion Assays. Native or heat-denatured type I collagen in 0.02 M acetic acid was added to 48-well plates at 40 μg per well and was air-dried to the bottom of the wells. Serum-free, concentrated, tumor-conditioned medium was added to some wells containing native collagen. This conditioned medium had been dialyzed into 50 mM Tris·HCl/10 mM $CaCl_2$/200 mM NaCl, pH 7.5 and was prefiltered to remove any high-molecular-weight ECM components ($\geq M_r$ 100,000). Aminophenylmercuric acetate (1 mM; Sigma) was added to conditioned medium (1 hr; 37°C) to activate collagenase activity, and TIMP-2 was added at 60 μg/ml as required. Conditioned medium from 10^7 M21 cells was added to each well in 100 μl of buffer and incubated for 24 hr at 37°C to promote collagenolysis. Melanoma cell adhesion to treated wells (1×10^5 cells per well) was assessed as described (16). For inhibition studies, cells were pretreated and incubated with purified mAbs at 20 μg/ml. After 1 hr, nonadherent cells were removed by gentle washing, and attached cells were enumerated with a hemacytometer.

Assessment of Cell Survival and Growth. Melanoma cells were seeded into 48-well tissue culture plates at 5×10^4 cells per well. To inhibit integrin ligation, some cells were pretreated with mAbs directed to $\alpha_2\beta_1$ (6F1) or $\alpha_v\beta_3$ (LM609). These cells were then added to culture wells together with mAb at a concentration of 80 μg per well. All cells were resuspended in 400 μl of cold collagen solution supplemented with FCS (2 or 10%) or Nutridoma (1%) as required. Cells resuspended in collagen without serum were previously maintained in serum-free Nutridoma. Collagen gels were overlaid with 200 μl of RPMI 1640 medium, and this medium was replaced every 2 days with fresh RPMI 1640 medium supplemented with FCS, Nutridoma, and/or mAb as required. Cells were extracted from collagen gels by the addition of clostridial collagenase (Worthington) at 0.2 mg per well (148 units/mg), and the cell number was determined with a hemacytometer. Viability was assessed with 0.2% trypan blue.

Analysis of Nuclear Chromatin. M21 cells or M21-L cells were seeded into 12-well tissue culture plates at 5×10^5 cells per well and suspended in 1-ml aliquots of dermal collagen. The cells were incubated for 4 days before extraction and staining with the DNA stain acridine orange (10 μg/ml in phosphate-buffered saline; Sigma). Cells were stained for 1–2 min, and nuclear chromatin was visualized with a fluorescent photomicroscope.

Analysis of DNA Fragmentation. M21 or M21-L cells were seeded into six-well tissue culture plates at 5×10^6 cells per well and suspended in 4-ml aliquots of dermal collagen. Three days later these cells were extracted from the collagen gels and resuspended in 0.5% Triton-100 lysis buffer. DNA was extracted by sequential treatment with phenol and phenol/chloroform before being precipitated in ethanol as described (19). DNA derived from either M21 or M21-L cells was analyzed in a 1.2% agarose gel, and the pattern of DNA fragmentation was visualized with ethidium bromide.

RESULTS

An α_v-Integrin Regulates Melanoma Cell Morphology in Dermal Collagen. To understand the association between $\alpha_v\beta_3$ expression and melanoma progression in the dermal environment (11, 12) we assessed the behavior of $\alpha_v(-)$ or $\alpha_v(+)$ melanoma cells embedded in dermal collagen. We selected M21 melanoma variants lacking α_v expression (M21-L), and some of these deficient cells were transfected with an α_v cDNA to restore $\alpha_v\beta_3$ expression (M21-L4 cells; ref. 12). To control for the effects of transfection we generated $\alpha_v(-)$ mock transfectants (M21-L12 cells). Selection and transfection did not alter the expression of other β1 integrins expressed by these cells. M21 and M21-L4 cells express comparable levels of $\alpha_v\beta_3$ (12).

Both $\alpha_v(+)$ and $\alpha_v(-)$ melanoma cells assumed a spread dendritic morphology after 24 hr in dermal collagen, in either the presence or absence of serum (Fig. 1 *A* and *B*), and this cell spreading could be abrogated by a mAb directed to $\alpha_2\beta_1$ (data not shown). However, after 24 hr, a progressive decrease in cell spreading occurred among the $\alpha_v(-)$ M21-L and M21-L12 cells (Fig. 1 *A* and *B*). In contrast, $\alpha_v(+)$ M21 and M21-L4 cells maintained their spread dendritic morphology throughout the 4-day assay (Fig. 1 *A* and *B*). Together these data implicate an α_v integrin in the regulation of cell morphology within these collagen matrices. Because M21 cell adhesion to native collagen is mediated by $\alpha_2\beta_1$ and not an α_v integrin (12, 14), these findings suggest that the melanoma cells alter the collagen to promote *de novo* ligation of the α_v integrin.

Melanoma-Mediated Degradation of Native Collagen Promotes $\alpha_v\beta_3$ Ligation. We demonstrated previously that α_v integrins do not mediate M21 cell adhesion to native collagen (12, 14). However, $\alpha_v\beta_3$ will ligate heat-denatured type I collagen (8). Because proteolysis will promote collagen denaturation at physiological temperatures (9), we tested whether the melanoma cells could expose cryptic $\alpha_v\beta_3$ sites by collagenolysis. We therefore looked for evidence of $\alpha_v\beta_3$ ligation to type I collagen that has been pretreated with M21-cell-conditioned medium containing collagenolytic activity. Both M21 and M21-L cells can directly degrade type I collagen (data not shown). As shown in Fig. 2*A Top* all cells, irrespective of α_v expression, adhered to native type I collagen. This adhesion could only be blocked with mAbs specific for β_1 or $\alpha_2\beta_1$ (Fig. 2*B*, *Top*). Denaturation of type I collagen by heating or by proteolysis caused profound changes in integrin-mediated adhesion. Thus, only $\alpha_v(+)$ cells (M21 or M21-L4) adhered significantly to the denatured ligand (Fig. 2*A*, *Middle* and *Bottom*), and this adhesion could be blocked by a mAb (LM609) directed to $\alpha_v\beta_3$ (Fig. 2*B*, *Middle* or *Bottom*). This $\alpha_v\beta_3$-dependent adhesion was accompanied by a loss in β_1 or $\alpha_2\beta_1$-dependent binding (Fig. 2*B*, *Middle* or *Bottom*). Addition of TIMP-2 to tumor-conditioned medium prevented collagenolysis and thereby restored adhesion by $\alpha_v(-)$ M21-L cells (Fig. 2*A*, *Bottom*, *Inset*). This observation is consistent with proteolysis by interstitial collagenase, which effectively degrades type I collagen and is inhibited by TIMP-2. Together these data indicate that melanoma-mediated proteolysis can disrupt

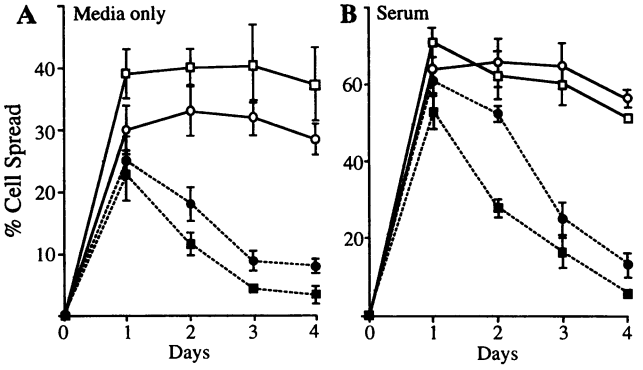

FIG. 1. An α_v-integrin regulates melanoma cell morphology in dermal collagen. Wild-type $\alpha_v(+)$ M21 cells (○), $\alpha_v(+)$ M21-L4 cells (□), $\alpha_v(-)$ M21-L cells (●), and $\alpha_v(-)$ M21-L12 cells (■) were maintained in dermal collagen in the absence (*A*) or presence (*B*) of 2% FCS. Cells within collagen gels were scored as either round or spread, and the results were expressed as average percentage of cell spread per high-powered field. Each point represents the mean (±SD) of three high-powered fields containing a minimum of 50 cells per field.

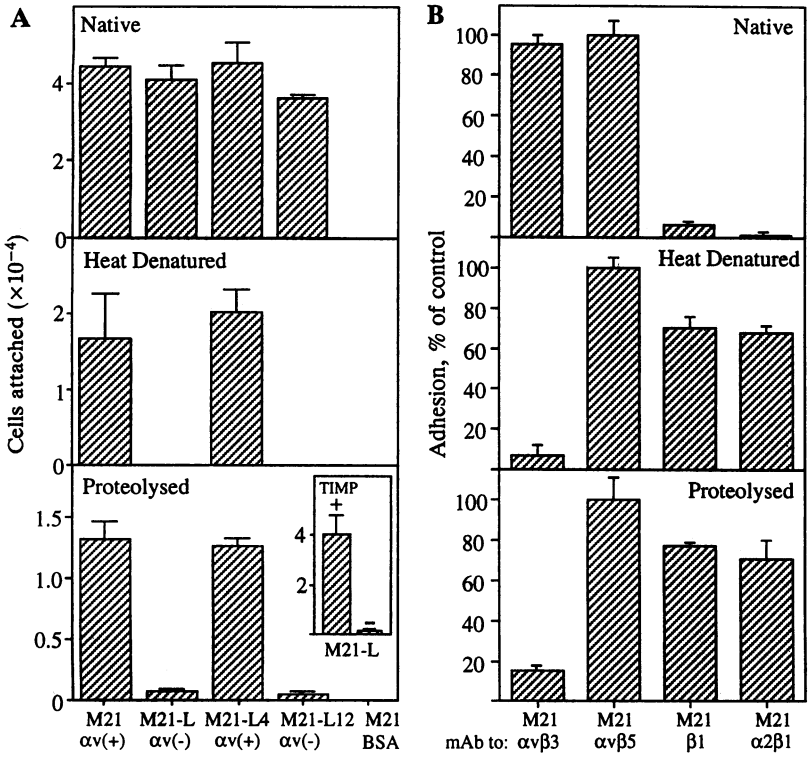

FIG. 2. Melanoma-mediated degradation of collagen promotes $\alpha_v\beta_3$ ligation. (*A*) M21 cells and α_v variants were added to wells coated with native, heat-treated, or proteolysed type I collagen and allowed to adhere for 1 hr. (*Inset*) M21-L cells were added to wells containing collagen pretreated with tumor-conditioned medium and inhibitor TIMP-2. (*B*) To inhibit integrin ligation, M21 cells were incubated with mAbs directed to $\alpha_v\beta_3$, $\alpha_v\beta_5$, or $\alpha_2\beta_1$. Each bar represents the mean (±SD) of three replicates. Results for antibody-inhibition studies are expressed as a percentage of the adhesion observed in the absence of antibody.

$\alpha_2\beta_1$-dependent binding to collagen while promoting direct ligation of $\alpha_v\beta_3$.

Integrin $\alpha_v\beta_3$ Promotes M21 Melanoma Cell Survival and Growth in Dermal Collagen. To test whether $\alpha_v\beta_3$ confers a significant survival advantage in the dermal ECM we assessed the long-term viability of α_v variants in dermal collagen gels. In the absence of exogenous growth factors, $\alpha_v(+)$ M21 cells were observed to maintain a constant viable cell number throughout the 6-day assay (Fig. 3*A*). However, $\alpha_v(-)$ M21-L cells only maintained viability 2 days before rapid and substantial cell death was observed (Fig. 3*A*). Comparable cell death could be induced in $\alpha_v(+)$ M21 by treatment with a function-blocking mAb (LM609) specific for $\alpha_v\beta_3$ (Fig. 3*A*). Treatment with mAb LM609 consistently induced a 30%–80% decline in M21 cell viability after 6 days in three-dimensional collagen. Antibody to $\alpha_2\beta_1$ (6F1), used under similar experimental conditions, did not promote a loss in cell viability (Fig. 3*B*), suggesting that rescue from cell death is a specific property of $\alpha_v\beta_3$. M21-L cells transfected with an α_v cDNA to restore $\alpha_v\beta_3$ expression (M21-L4) were effectively rescued from cell death (Fig. 3*B*). M21 cells and the α_v variants all maintained long-term viability on two-dimensional type I collagen (data not shown) or other adhesive proteins (20). This finding demonstrates that there is no intrinsic difference in the ability of these cells to survive and indicates that the three-dimensional nature of the collagen is important in regulating cell viability.

Addition of insulin (Nutridoma-SP) or serum to collagen matrices promoted long-term proliferation of both $\alpha_v(-)$ and $\alpha_v(+)$ cells. Growth of M21 cells and M21-L cells was identical for the first 5 days of culture, and no significant cell death was observed in $\alpha_v(-)$ cells (Fig. 4 *A* and *B*). This finding indicates that exogenous growth factors can prevent cell death in our $\alpha_v(-)$ cells. However, during the last 6 days of culture, $\alpha_v(+)$ M21 cells were able to sustain a significantly higher growth rate than $\alpha_v(-)$ M21-L cells (Fig. 4 *A* and *B*). A reduction in the amount of serum added to the collagen from 10% to 2% increased the disparity in growth between $\alpha_v(+)$ and $\alpha_v(-)$ cells (Fig. 4*C*). These data suggest that ligation of the α_v subunit may be most advantageous for

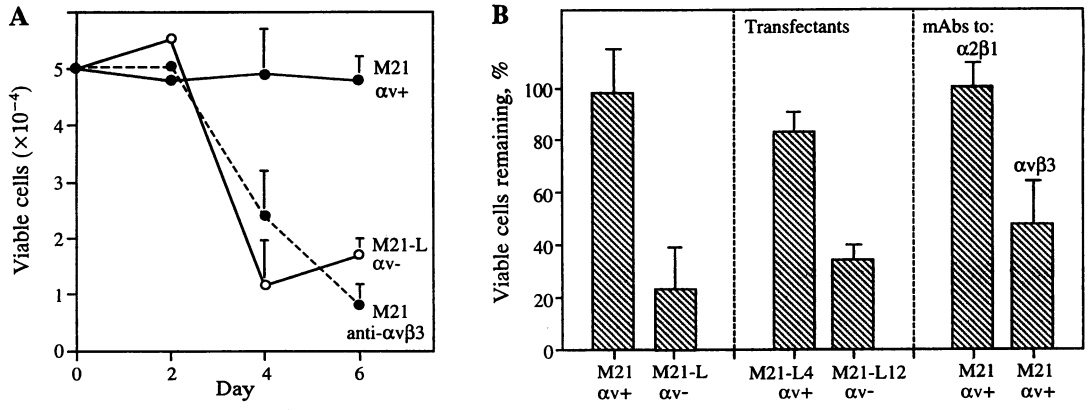

FIG. 3. Integrin $\alpha_v\beta_3$ promotes M21 melanoma cell survival in dermal collagen. M21 cells and α_v variants were maintained in dermal collagen (5×10^4 cells per 400 μl of gel) in the absence of exogenous growth factors. To inhibit integrin ligation, some cells were incubated with mAbs directed against $\alpha_2\beta_1$ or $\alpha_v\beta_3$. At 48-hr intervals cells were extracted, and the viable cell number was determined. Each data point represents the mean (±SD) of three replicates.

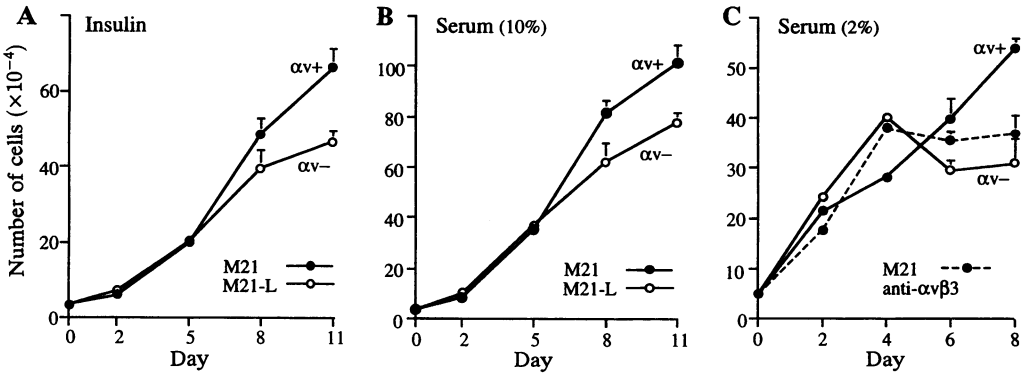

FIG. 4. Integrin $\alpha_v\beta_3$ promotes melanoma cell growth in dermal collagen. M21 cells or $\alpha_v(-)$ M21-L cells were maintained in dermal collagen (5×10^4 cells per 400 μl of gel) in the presence of insulin (*A*) or serum at 10% (*B*) or 2% (*C*). To inhibit integrin ligation, some of the cells were incubated with a mAb directed to $\alpha_v\beta_3$. At 48- to 72-hr intervals cells were extracted, and viable cell number was determined. Each data point represents the mean (±SD) of three replicates.

growth when the supply of growth factors is limiting. A role for $\alpha_v\beta_3$ in the regulation of this growth is indicated by the ability of mAb LM609 to nullify the α_v-dependent growth advantage (Fig. 4*C*). M21 cells and associated $\alpha_v(-)$ variants all had comparable long-term growth rates when grown under normal culture conditions in the presence of 10% FCS (12).

Cell Death in $\alpha_v\beta_3$-Deficient Cells Is by Apoptosis. We have demonstrated that $\alpha_v(-)$ cells maintained in collagen gels undergo significant cell death (Fig. 3 *A* and *B*). This cell death was associated with cell shrinkage, plasma membrane blebbing, and the appearance of apoptotic bodies (Fig. 5 *B* and *C*). In addition, these $\alpha_v(-)$ cells showed evidence of chromatin condensation, nuclear segmentation, and oligonucleosomal DNA fragmentation (Fig. 5 *E–G*). All these findings are indicative of death by apoptosis (21). In contrast to the $\alpha_v(-)$ cells, $\alpha_v(+)$ M21 cells maintained in collagen and examined at the same time point had a viable appearance, frequently displayed a spread dendritic morphology, had normal nuclear chromatin, and showed no evidence of significant DNA fragmentation (Fig. 5 *A*, *D*, and *G*). Together these data demonstrate that the loss of $\alpha_v(-)$ cell viability in dermal collagen is due to apoptosis and confirms that ligation of an α_v integrin within this matrix rescues cells from this process.

DISCUSSION

Anchorage-independent growth is recognized as a hallmark of the transformed cell. However, in this study, we demonstrate a mechanism of tumor cell growth that incorporates both anchorage-dependent survival and growth. Specifically, we show that ligation of the vitronectin receptor $\alpha_v\beta_3$, within a three-dimensional dermal collagen matrix, suppresses apoptosis and promotes melanoma cell growth. We propose that this mechanism may be responsible for the association between $\alpha_v\beta_3$ expression and melanoma progression (10–12). In

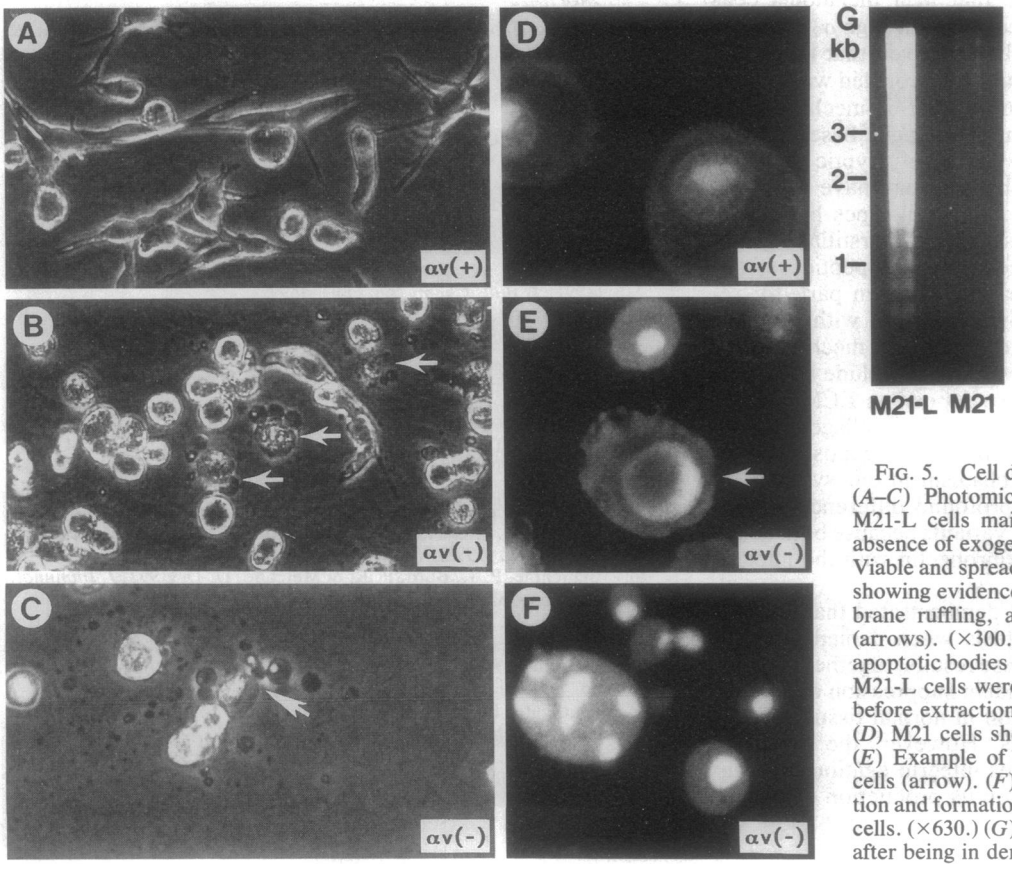

FIG. 5. Cell death in $\alpha_v(-)$ cells is by apoptosis. (*A–C*) Photomicrographs of M21 cells or $\alpha_v(-)$ M21-L cells maintained in dermal collagen in the absence of exogenous growth factors for 6 days. (*A*) Viable and spread M21 cells. (×300.) (*B*) M21-L cells showing evidence of cell rounding, shrinkage, membrane ruffling, and formation of apoptotic bodies (arrows). (×300.) (*C*) M21-L cell fragmenting into apoptotic bodies (arrow). (×400.) (*D–F*) M21 cells or M21-L cells were maintained in collagen for 4 days before extraction and staining with acridine orange. (*D*) M21 cells showing normal nuclear morphology. (*E*) Example of chromatin condensation in M21-L cells (arrow). (*F*) Example of the nuclear segmentation and formation of apoptotic bodies seen in M21-L cells. (×630.) (*G*) DNA fragmentation in M21-L cells after being in dermal collagen for 3 days.

this regard, we previously reported that $\alpha_v(-)$ M21-L and M21-L12 cells have a dramatically reduced tumorigenicity in the skin of nude mice compared with $\alpha_v(+)$ M21 and M21-L4 cells (12).

Suppression of apoptosis has been linked to the ligation of a variety of survival factors including growth factors (22), hormones (23), and cytokines (24). Our findings imply that ligation to the ECM will also suppress apoptosis. Current paradigms suggest that different tissues produce unique sets of factors that ensure the survival of resident cell types, whereas misplaced cells will be deprived of requisite factors (25). Metastatic cells may circumvent this protective mechanism by producing their own survival factors. It is conceivable that $\alpha_v\beta_3$ expression on malignant melanoma cells may also function in this capacity by promoting aberrant survival in the dermal ECM.

Our data indicate that $\alpha_v\beta_3$ confers a significant growth advantage to cells in the presence of insulin or serum. Evidently, suppression of apoptosis will ensure that more cells are available for mitogenic growth stimulation. However, ligation of $\alpha_v\beta_3$ may also generate a comitogenic signal that acts in concert with insulin or serum growth factors to optimize cell growth (26). Our findings suggest, however, that $\alpha_v\beta_3$ ligation only produces a significant growth or survival advantage when other survival factors are limiting. Thus, it is clear that α_v-deficient cells can be rescued from cell death if sufficient amounts of insulin or serum growth factors are present. This observation is consistent with previous reports linking suppression of apoptosis with ligation of growth factors (25). Together these observations imply that ligation of $\alpha_v\beta_3$ will be most relevant to tumor cell survival and growth before angiogenesis or in poorly vascularized regions of the tumor where the supply of survival factors may be limiting.

Several observations suggest that the melanoma cells used in this study alter the dermal collagen to promote $\alpha_v\beta_3$ ligation. Thus, we demonstrate that M21 melanoma cells initially adhere to native type I collagen through $\alpha_2\beta_1$ and do not adhere through $\alpha_v\beta_3$. We also observed that initial cell spreading within the collagen could be abrogated with a mAb to $\alpha_2\beta_1$ (data not shown). We provide a mechanism to account for $\alpha_v\beta_3$ ligation within the collagen based on collagenolysis and concomitant exposure of cryptic adhesive sites. We believe that this mechanism may have a general biological significance because many cell types have been shown to denature collagen by secreting interstitial collagenase (27) or by producing a spectrum of telopeptidases (9). Conceivably this mechanism may account, in part, for reports linking increased cell growth in ECM with protease secretion (27, 28). Although we propose a mechanism of ligation based on degradation, we do not exclude the possibility that the tumor cells will also deposit an ECM component, such as fibronectin, which can bind collagen and be ligated by $\alpha_v\beta_3$. However, if fibronectin deposition and ligation is indeed a primary mechanism in our system, we would not expect to see such profound differences in the adhesion and spreading of our α_v variants because both $\alpha_v(-)$ and $\alpha_v(+)$ M21 cells can ligate fibronectin with the integrin $\alpha_5\beta_1$ (29).

Recently, Meredith *et al.* (30) demonstrated that primary endothelial cells undergo apoptosis when denied integrin-dependent adhesion to ECM. These results, together with our own, suggest that integrin-dependent suppression of apoptosis may be a general phenomenon in normal tissue homeostasis, as well as tumorigenesis. However, the extent to which a transformed cell requires integrin ligation to avoid apoptosis may vary, depending on its production of autologous survival factors.

Interesting questions remain to be answered with respect to the signaling pathways initiated by $\alpha_v\beta_3$ ligation. However, the observations that integrin $\alpha_v\beta_3$ is expressed on the most proliferative melanoma cells in humans (11) and provides a growth advantage to human melanoma cells in the nude mouse (12) suggest that this integrin is capable of initiating signals relevant to the outgrowth of human melanoma. We present evidence that an important component of this signaling event may be the suppression of apoptosis. Indeed, this mechanism may contribute to the dormant survival of such cells in the dermal environment and allow for subsequent outgrowth and metastasis when other growth stimuli become available.

We acknowledge the excellent technical assistance provided by Shelly Denton. This work was supported by National Cancer Institute Outstanding Investigator Grant CA42508 (R.A.R.), and by Grants CA45726 and CA50286 (D.A.C.). Further support was provided by a grant from the Tobacco Related Disease Program from the state of California (D.A.C.). D.A.C. is a recipient of a Faculty Research Award from the American Cancer Society. This is the Research Institute of Scripps Clinic Manuscript 8270-IMM.

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