The Laryngoscope
Lippincott Williams & Wilkins, Inc., Philadelphia

Integrins in Head and Neck Squamous Cell Carcinoma Invasion

Orville H. Dyce, MD; Amy F. Ziober, JD; Randal S. Weber, MD; Kaoru Miyazaki, PhD; Samir S. Khariwala, MD; Michael Feldman, MD, PhD; Barry L. Ziober, PhD

Objective: **To relate the invasive properties of different squamous cell cancer cell lines to the function and expression of the integrins.** ***Study Design:*** **A series of in vitro and in vivo experiments were designed to assess and compare integrin expression and function in two different head and neck squamous cell carcinoma cell lines.** ***Methods:*** **Invasive properties of two squamous cell carcinoma cell lines (UM-SCC-1 and JHU-022-SCC) were assessed using an in vitro artificial matrix assay as well as an in vivo system with orthotopically implanted tumor cells in mice. Whole cell and surface expression levels of integrin subunits (alpha2, alpha3, alpha5, alpha6, beta1, and beta4) were determined for each cell line using Western blot analysis and flow cytometry. We compared the ability of JHU-022-SCC and UM-SCC-1 cells to bind the extracellular matrix elements collagen IV, fibronectin, laminin 5, and laminin10 using an in vitro adhesion assay. Contributions of the different integrins to the adhesive properties were determined by selective antibody blocking of different subunits.** ***Results:*** **The UM-SCC-1 cell line is 50% more invasive in vitro and displays a greater propensity for perineural and lymphatic invasion in vivo. The UM-SCC-1 cells exhibited greater adherence to fibronectin than JHU-022-SCC cells. Alpha6 and beta4 expression is approximately twofold greater in the JHU-022-SCC cells. Alpha2, alpha3, and beta1 expression appears to be upregulated in UM-SCC-1 cells.** ***Conclusion:*** **The UM-SCC-1 carcinoma cells are more invasive than JHU-022-SCC cells and may be related to differential expression of the integrins alpha6beta4, alpha3beta1, and alpha2beta1.**
Key Words: **Integrin, head and neck cancer, laminin, collagen IV, fibronectin.**

Laryngoscope, **112:2025–2032, 2002**

From the Departments of Otorhinolaryngology (O.H.D., A.F.Z., R.S.W., S.S.K., B.L.Z.), Pathology (B.L.Z.), and Radiation Oncology (B.L.Z.), University of Pennsylvania Medical Center, Philadelphia, Pennsylvania, U.S.A.; and the Division of Cell Biology (K.M.), Kihara Institute for Biological Research, Yokohama City University, Yokohama, Japan.

Editor's Note: This Manuscript was accepted for publication June 11, 2002.

Send Correspondence to Barry L. Ziober, PhD, Department of Otorhinolaryngology, 5 Ravdin, University of Pennsylvania Medical Center, Philadelphia, PA 19104, U.S.A. E-mail: bziober@mail.med.upenn.edu

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) accounts for the largest single group of cancers of the upper aerodigestive tract. Spread of these tumors can occur through direct extension to adjacent structures, lymphatic invasion with involvement of regional lymph node basins, and perineural invasion. Perhaps not as well recognized as other modalities, perineural spread is also important because its presence portends a worse prognosis.[1]

Despite histological similarities, head and neck squamous cell carcinomas exhibit different biological behaviors. Whereas some tumors are more aggressive with relatively early lymphatic and perineural involvement, these remain late findings in less aggressive tumors. Integrins may play a role in the differential behavioral patterns of head and neck squamous cell carcinomas.

Integrins are protein heterodimers composed of noncovalently associated transmembrane alpha and beta subunits. They can be found intracytoplasmically or along the cell surface. To date, approximately 16 alpha and 8 beta subunits have been identified.[2] These subunits combine to form receptors that are able to bind components of the extracellular matrix.

Integrins have important roles in multiple biological processes including cellular aggregation, growth, differentiation, migration, and apoptosis.[3] More recently, they have been shown to play significant roles in malignant tumor migration, extension, and metastasis.[3–6] We sought to investigate whether the differential functional expression of integrins could account for the different behavioral characteristics of HNSCC.

MATERIALS AND METHODS

Cell Culture and Materials

The human squamous cell cancer cell line UM-SCC-1 (from the University of Michigan) was cultured on 10-cm culture plates using Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovine serum (FBS). A second squamous cell cancer cell line, JHU-022-SCC (kindly provided by Wayne Koch, Johns Hopkins University), was cultured using Roswell Park Memorial Institute (RPMI) medium supplemented with 10% FBS and 1% glutamate. Cell cultures were harvested at 70% to 80% confluence using a short, 2.0-mL trypsin treatment (0.25% trypsin with

1.0 mmol/L ethylenediamine tetra-acetic acid [EDTA]). The addition of 5.0 mL DMEM neutralized the trypsin. Centrifugation for 2 minutes at 500*g* (4°C) allowed separation of the cells.

Antibodies against integrin subunits used for the adhesion assay included mouse monoclonal antibodies to alpha2 (P1E6, Chemicon), alpha3 (P1B5, Chemicon), alpha5 (P1D6, Chemicon), alpha6 (GoH3, Pharmingen), beta1 (6S6, Chemicon), and beta4 (ASC-3, Chemicon). Antibodies against integrin subunits used for Western blot analysis (included alpha2 (sc-6586, Santa Cruz), alpha3 (sc-6592, Santa Cruz), alpha5 (Transduction Laboratories), alpha6 (A33, kindly provided by Arnoud Sonnenberg of the Netherlands Cancer Institute), alphaV (VNR 139, Chemicon), beta1 (HB1.1, Chemicon), and beta4 (439-9B, Pharmingen). Antibodies against integrin subunits used for fluorescence-activated cell sorter (FACS) analysis included alpha2 (AK-7, Pharmingen), alpha3 (C3 II.1, Pharmingen), alpha5 (IIA1, Pharmingen), alpha6 (GoH3, Pharmingen), alphaV (VNR139, Chemicon), alphaVbeta6 (E7P6, Chemicon), beta1 (6S6, Chemicon), beta3 (B3A, Chemicon), and beta4 (ASC-3, Chemicon).

Migration Assay

We measured tumor cell migration along purified extracellular matrix (ECM) components collagen IV and laminin 5 (provided by Kaoru Miyazaki, PhD, Yokohama City University), using a modified Boyden chamber (Neuroprobe, Bethesda, MD). An 8-μm filter was precoated with the study ligand at a concentration of 0.5 μg/mL for 1 hour. The lower well of the Boyden chamber was filled with serum-free medium containing 0.1% bovine serum albumin (BSA). Fifty microliters of cell suspension was added to the upper chamber and then incubated for the indicated time at 37°C. The tumor cell suspensions were also incubated with antibodies directed against different integrin subunits. Cells on the top of the filter were removed by wiping, and the filter was fixed in 1% formaldehyde in phosphate-buffered saline (PBS). Migrated cells were then stained with 1% crystal violet, and nine randomly chosen fields from triplicate wells were counted at original magnification $\times$400.

Orthotopic Mouse Model

One hundred microliters of PBS containing 5 million tumor cells were injected into the cervical subcutaneous tissues of the 4-week-old athymic nude female mice (Harlan Sprague-Dawley). Eight mice were injected with UM-SCC-1 cells, and seven mice were injected with the JHU-O22-SCC cells. The mice were killed at 30 days.

All specimens were harvested in an identical fashion. Five coronal sections of the lower jaw and cervical region of each of the mice were harvested in an anterior to posterior direction. An experienced, board-certified pathologist specializing in head and neck tumors systematically evaluated representative slides prepared from each of the specimens in a blinded fashion. The presence or absence of perineural invasion was determined. During histological evaluation, a tally was kept from each specimen for evidence of lymphatic and vascular invasion.

Adhesion Assay and Blocking Assay

The ECM components studied were collagen IV, fibronectin, vitronectin, and laminin 1 (all from Becton Dickinson); laminin 5 (conditioned medium); and laminin 10 and laminin 11 (Gibco). Subunits of interest included alpha2, alpha3, alpha5, alpha6, alphaV, beta1, and beta4. Seventy-five microliters of each ligand at a concentration of 10 μg/mL was used to coat wells of Immulon-4 (Dynatech Laboratories) enzyme-linked immunosorbent assay (ELISA) 96-well plates and incubated for 1 hour at 37°C. One percent BSA was the negative control. Excess ligand was aspirated, and each well was washed with PBS. Two hundred microliters DMEM with 0.1% BSA was added to each test well and incubated for 1.5 hours at 37°C. Excess DMEM was aspirated, and 2×10^5 cells were incubated in each test well for 35 minutes at 37°C.

For the antibody inhibition assay, an equal volume of the blocking antibody was added to the cell solution to achieve a final antibody concentration of 10 μg/mL and incubated on ice for 1 hour before adding the cell suspension to each microwell.

Five washes with 200 μL PBS were followed by fixation with 100 μL 1% formaldehyde for 15 minutes. The formaldehyde was aspirated, and the adherent cells were stained for 20 minutes with 60 μL per well of 2% crystal violet in PBS. The plates were washed with distilled H_2O until all BSA wells were clear. The wells remained inverted overnight. The cells were lysed with 100 μL 2% SDS per well for 30 minutes, and the optical density (OD) of each well was 562 nm. We applied Student *t* test to compare the ability of JHU-022-SCC and UM-SCC-1 cells to adhere to the substrate before and after incubation with blocking antibodies.

Western Blot Analysis

Seventy percent confluent cell cultures on 10-cm plates were washed with calcium-free PBS. Two hundred microliters of lysis buffer (0.5% Triton, 50 mmol/L Tris [pH 7.5], and 1.0 mmol/L $MgCl_2$ with fresh 1.0 mmol/L phenylmethylsulfonyl fluoride [PMSF] and 1.0 mM/L N-ethylmaleimide [NEM]) were added to each plate and swirled gently for 30 minutes. A cell lifter was used to scrape the plate, and the whole cell lysate was placed in a Microfuge tube and centrifuged for 5 minutes at 14,000*g*. The supernatant was aspirated, and aliquots of 30 μg of protein were obtained by standardizing the samples using the Pierce assay.

Thirty micrograms of whole cell lysate from each cell were prepared by adding sample buffer and heating at 95°C for 3 minutes. The samples were cooled to room temperature, loaded onto 7% resolving sodium dodecyl sulfate polyacrylamide gels (SDS-PAG), and run at 130 V for approximately 60 minutes. Semidry mini protein transfer to membrane filters (Immobilon-P membranes) was performed at 13 V for 31 minutes with a maximum current limit set at 5.5 mA/cm^2.

Enhanced chemiluminescence (ECL) detected proteins along the Immobilon-P membranes. The membranes were first blocked overnight using a 5% blocking solution (5% powdered milk in Dulbecco's-PBS with 0.1% Tween). Three 10-minute washes (0.1% Tween in Dulbecco's-PBS) were followed by incubation for 1 hour with the primary antibody diluted with the blocking solution. A second set of three washes was followed by an hour-long incubation with the appropriate horseradish peroxidase (HRP) conjugated secondary antibody. A third set of washes was followed by incubation with the detection solution (Amersham) for 1 minute. The membranes were then exposed to x-ray film for signal detection.

Fluorescence-Activated Cell Sorter Analysis

Flow cytometric analysis was performed on cells from each of the two cell lines. Cultured cells of 70% to 90% confluence were harvested by brief trypsinization (0.25% trypsin and 1 mM/L EDTA) for approximately 2 minutes. One million cells were resuspended in 200 μL phosphate-buffered saline–fetal calf serum (PBS-FCS).

Primary antibody was added to each cell solution to a final antibody concentration of 2.5 μg/mL. The cells were incubated on ice for 1 hour followed by three 200-μL PBS-FCS washes. The secondary antibody was added, and the solution was incubated on ice for 30 minutes. Four 200-μL washes with PBS-FCS were performed. Propidium iodide was added to a final concentration of 1 μg/mL. The cells were then resuspended in 500 μL PBS-FCS and loaded into the FACS machine.

RESULTS

Migration Assay

In Figure 1A and B, migration of the tumor cell lines JHU-022-SCC and UM-SCC-1 on type IV collagen (10 μg/mL) is compared. There was no difference in migration along collagen IV. The JHU-022-SCC cell line demonstrated approximately 50% diminished migratory capacity on laminin 5 compared with UM-SCC-1 cells.

Adhesion Assay

The optical density obtained for collagen IV binding was used as a reference point and set at 100%. Adhesion of the cell lines to different ECM components was compared with collagen IV and expressed as a percentage. These results are illustrated graphically in Figure 2. No differences in collagen IV adhesion were seen for JHU-022-SCC versus the UM-SCC-1 cell lines. A difference in adhesion to fibronectin was evident between the cell lines (by Student t test, $P \leq .004$). Neither cell line demonstrated significant adhesion to BSA, vitronectin, or laminin 1 (Fig. 2). However, adhesion to laminin 5 as well as laminin 10 and laminin 11 ECM components was comparable to or superseded that of collagen IV.

Incubating the cell lines with blocking antibodies to the beta1 subunit decreased adhesion of both cell lines to collagen IV (by Student t test, $P \leq .007$ for UM-SCC-1 and $P \leq .004$ for JHU-022-SCC) (Fig. 3A) as well as fibronectin (by Student t test, $P \leq .004$ for UM-SCC-1 and $P \leq .007$ for JHU-022-SCC) (Fig. 3B). Exposure of UM-SCC-1 cells to alpha2 and alpha5 blocking antibodies decreased adhesion to collagen IV ($P \leq .011$) (Fig. 3A) and fibronectin ($P \leq .05$) (Fig. 3B), respectively. The JHU-022-SCC cells exhibited decreased binding to collagen IV (Fig. 3A) as well as fibronectin (Fig. 3B) after incubation with blocking antibodies to the alpha2 ($P \leq .007$) and alpha5 ($P \leq .019$) chains, respectively. In addition, blocking antibodies to the alpha3, alpha6, beta1, and beta4 integrin subunits did not decrease cellular adhesion to laminin 10 and laminin 11 (Fig. 4).

Western Blot and Enhanced Chemiluminescence Analysis

Semiquantitative analysis of whole cell expression of the different integrin subunits using Western blot analysis with ECL immunodetection revealed increased whole cell expression of alpha2, alphaV, and beta1 for the UM-SCC-1 cells. Representative Western blots comparing alpha6 and alphaV expression in the UM-SCC-1 versus JHU-022-SCC cell lines are shown in Figure 5. Because of the number of integrins compared, we present the findings in summary form in Table I. Similar expression levels between the cell lines were detected for the alpha3 subunit (Table I).

Fluorescence-Activated Cell Sorter Analysis

Quantitative analysis of cell surface integrin expression reveals levels of alpha6 and beta4 subunits for the JHU-022-SCC cell line approximately twice that of UM-SCC-1 cells. This is illustrated in Table II. Conversely, cell surface expression of alpha2, alpha3, alpha5, and alphaV-beta6 was doubled in the UM-SCC-1 cells. Significant differences in expression were not seen for the alphaV, beta1, or beta 3 subunits (Table II).

In Vivo Assay and Orthotopic Mouse Tumor Transplant

All mice transplanted with tumor cells developed tumors. Growth rates exhibited wide variation and, overall, were similar between the two groups of tumors. Significant differences in tumor size were not seen with mice transplanted with the UM-SCC-1 tumor cells versus the JHU-022-SCC cells (111 $\pm$ 31 cm^2 vs. 107 $\pm$ 53 cm^2, respectively (Table III).

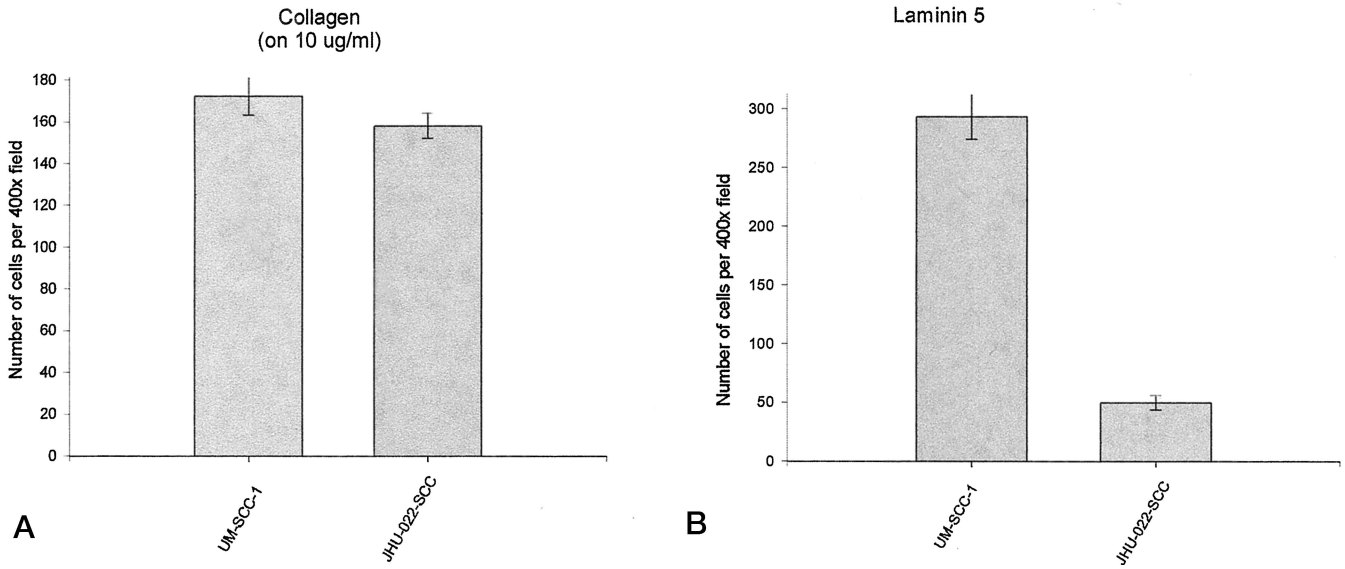

Fig. 1. Comparison of the migratory capabilities of the cell lines UM-SCC-1 and JHU-022-SCC on (**A**) 10 μg/mL collagen IV versus (**B**) 10 μg/mL laminin 5. JHU-022-SCC migration along the laminin 5 substrate is diminished compared with UM-SCC-1.

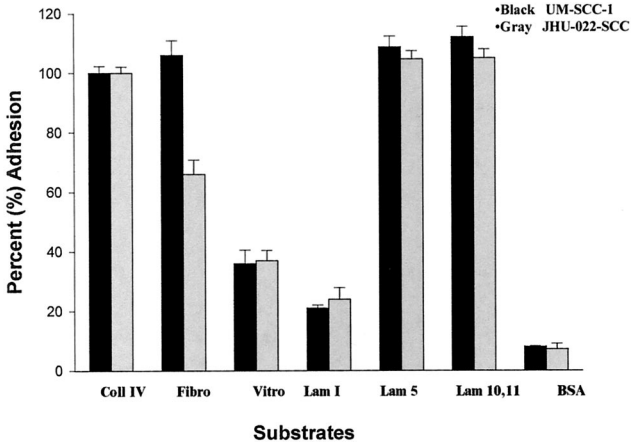

Fig. 2. Adhesion of the UM-SCC-1 cells to microwells coated with 10 μg/mL of the extracellular matrix substrates collagen, fibronectin, vitronectin, laminin 1, laminin 5, and laminin 10 and laminin 11 with bovine serum albumin as a control. Adhesion to the different substrates is compared with collagen and expressed as a percentage of binding to collagen. JHU-022-SCC exhibited diminished adhesion to fibronectin compared with UM-SCC-1 cells (Student *t* test, $P \leq .004$).

Vascular invasion was present on histological evaluation of tumors from the both the UM-SCC-1 and the JHU-022-SCC injected mice (Table III). Regional metastatic lymphadenopathy, although more evident on examination of tumor specimens from mice injected with the UM-SCC-1 cells, was also present in tumors specimens from the JHU-022-SCC injected mice (Table III). Perineural invasion with tumor growth within the nerve sheath (Table III) was clearly seen in a UM-SCC-1 derived tumor. We found no evidence of perineural spread in tumors from the JHU-022-SCC cell lines at 30 days.

DISCUSSION

Squamous cell carcinoma of the head and neck is a heterogeneous condition with a variety of clinical presentations. Although microscopic similarities are apparent between tumor types, their behavior can exhibit wide variability. Some tumors are characterized by slow enlargement with late lymphatic involvement, and others exhibit more aggressive behavior with early lymphatic spread, rapid growth, and varying propensities for extension to adjacent structures such as nerves and blood vessels. Anatomical factors such as the density of lymphatics in the site of origin partially explain these differences. It is likely that factors inherent to tumors contribute as well.

Specific patterns of integrin expression may play a role in these differences. Integrins are heterodimeric molecules expressed on a cell's surface. They are required for many of cellular processes. These molecules have demonstrated importance in cell growth and differentiation,[7] tissue repair,[8] intracellular signaling,[7] and tumorogenesis.[3] Differences in integrin function have already been noted in malignant versus nonmalignant tissue types. Downregulation of collagen IV and laminin binding has been demonstrated in carcinomas when compared with their normal epithelial cell counterparts.[9]

Comparing tumors with different behaviors allows correlation of tumor invasiveness with integrin expression and function. The cell lines were chosen based on previous studies that demonstrated contrasting invasive properties of UM-SCC-1 derived tumors versus those from JHU-022-SCC cells. Previous work by Simon et al.[10] in 1998 reported muscular, vascular, and perineural invasion by UM-SCC-1 derived tumors but not JHU-022-SCC tumors 30 days after both were injected into the cervical subcutaneous tissues of mice. Further evidence that the UM-SCC-1 and JHU-022-SCC tumor lines differed in properties came from earlier unpublished observations of

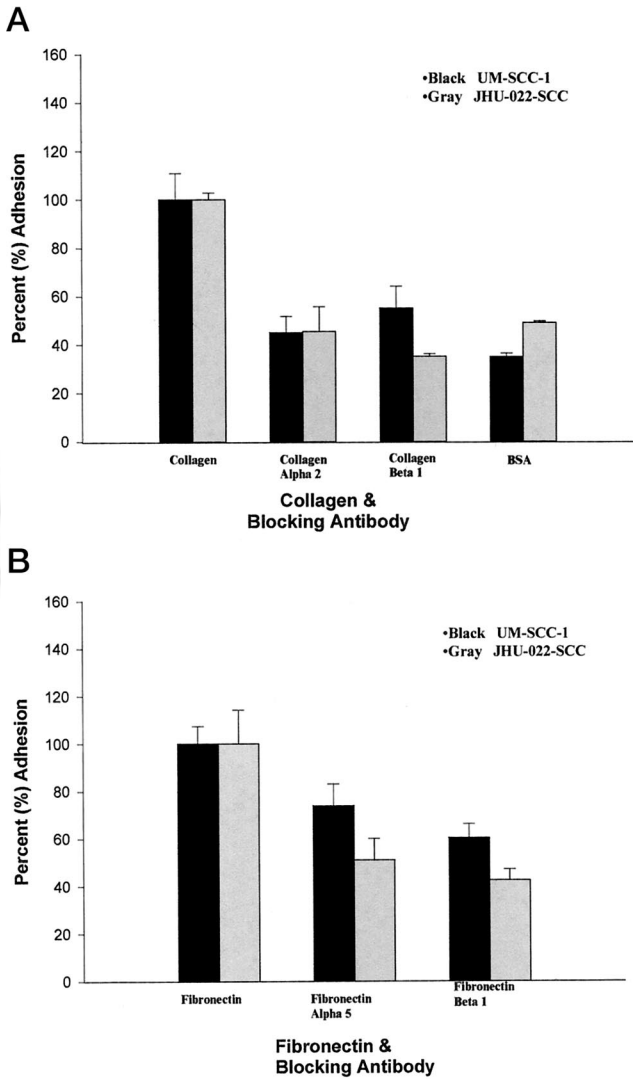

Fig. 3. (**A**) A reduction in binding to collagen by UM-SCC-1 cell in the presence of alpha2 (Student *t* test, $P \leq .011$) and beta1 ($P \leq .007$) inhibitory antibodies. JHU-022-SCC exhibited decreased adhesion to collagen in the presence of alpha2 ($P \leq .007$) and beta1 ($P \leq .004$) inhibition. (**B**) Decreased adhesion of UM-SCC-1 cells to fibronectin in the presence of alpha5 ($P \leq .05$) and beta1 ($P \leq .004$) chain inhibition. A decrease in adhesion to fibronectin is exhibited by the JHU-022-SCC after exposure to alpha5 ($P \leq .019$) and beta1 ($P \leq .007$) inhibition antibodies. Bovine serum albumin was used as a control.

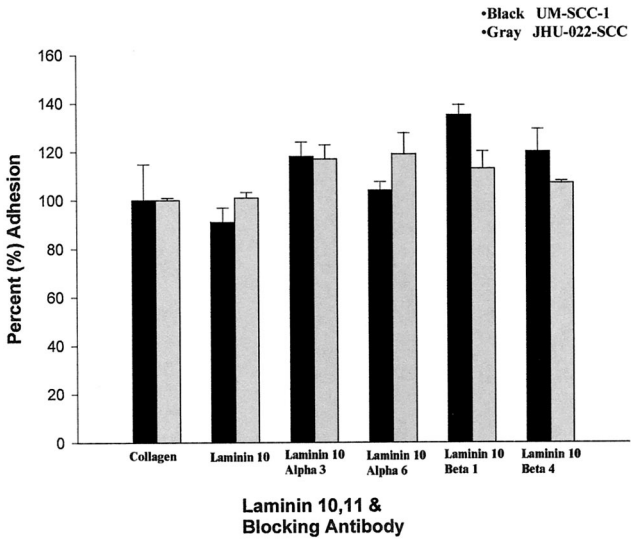

Fig. 4. Adhesion of UM SCC1 and JHU 022 cells lines to the laminin 10 and laminin 11 substrate remained unchanged despite the presence of blocking antibodies to the integrin subunits alpha3, alpha6, beta1, and beta4 ($P > .05$).

increased ability of the UM-SCC-1 cell to invade an artificial matrix system.[10]

We compared tumor cell migration and adhesion using in vitro systems, as well as tumor invasion using the previously established in vivo model. Differences in behavior of the tumors could be compared with the expression of integrins as determined by Western blot or FACS analysis. Of particular interest to us were the receptors allowing tumor cell interaction with laminin-5. Multiple tumor types including HNSCC mucosal tumors produce laminin 5.[11–13] Overexpression of laminin 5 has been associated with more invasive tumor fronts,[13] and an increasingly irregularly staining pattern for laminin 5 has been observed as tumors progressed from dysplasia to frank carcinoma.[14] Ono et al.[15] have described an association between the presence of laminin 5 and increased tumor invasiveness. In addition, more aggressive tumors that have exhibited perineural invasion have demonstrated increased laminin 5 staining.[16] Tumor cell interaction with laminin 5 is likely to play a major role in tumor aggressiveness and progression.

The JHU-022-SCC cells exhibited diminished motility along laminin 5 compared with the UM-SCC-1 cells (Fig. 1B). It is unlikely that the differential was due to a motility defect because migration of JHU-022-SCC along collagen IV was unaffected (Fig. 1A). More likely, interactions with laminin 5, mediated through unique receptor mechanisms, accounted for these findings. Classically described receptors for laminin 5 include alpha2beta1, alpha3beta1, and alpha6beta4. The alpha3 and alpha2 receptors subunits have demonstrated roles in cellular motility.[17]

The alpha3beta1 receptor is probably required for cell spreading on laminin 5 and may contribute to weaker interactions with other ECM components.[17,18] Cellular motility may be regulated through linkages with CD151 and phosphatidylinositol 4-kinase.[19] The alpha2beta1 receptor has recently been described as a receptor for laminin 5 and may also play a role in cell motility on this.[20]

Alpha3 and alpha2 subunits were both underexpressed by the JHU-022-SCC cell line compared with UM-SCC-1 cells. The reduced subunit levels probably indicate decreased alpha3beta1 and alpha2beta1 integrin levels limiting interaction with laminin 5. This difference in cell surface expression was confirmed by FACS analysis and may help to explain the differential motility patterns observed.

As an alternative, the alpha6beta4 receptor could have contributed to the lack of migration of the JHU-022-SCC cells along laminin 5. The alpha6beta4 receptor is required during intercellular interactions, as well as to promote cellular stability.[21] The alpha6beta4 integrin is thought to be primarily responsible for cell anchoring to laminin 5 through the formation of hemidesmosomes.[22,23] We found lower levels of the alpha6 and beta4 subunits in the more aggressive UM-SCC-1 cell line by both Western blot and FACS analysis. These findings may suggest a lack of the dermal-epidermal adhesive structure provided by hemidesmosome formation in this cell line. The lack of the alpha6beta4 receptor has already been associated with increased tumor progression[23,24] and poorer tumor differentiation.[6] In our studies, the lack of this receptor may have contributed to a more migratory and aggressive phenotype.

Studies of in vitro adhesion demonstrated similar binding for both cell lines on collagen IV, laminin 5, and

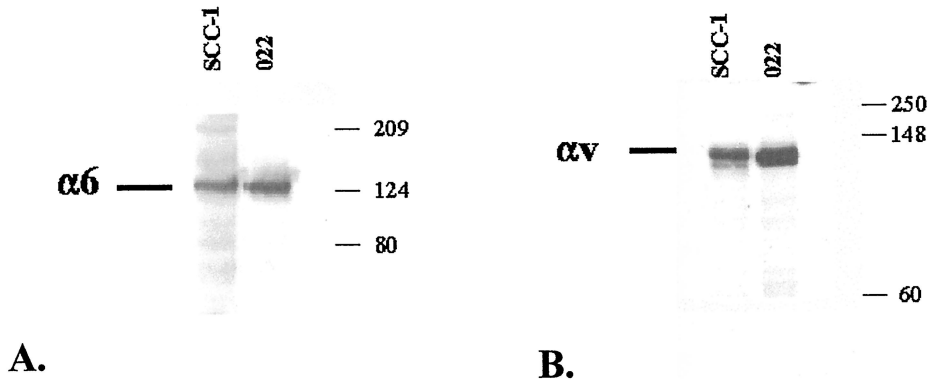

Fig. 5. Western blot comparisons of JHU-022-SCC versus UM-SCC-1 (**A**) alpha6 and (**B**) alphaV expression. Shown is a representative Western blot analysis detecting whole cell expression of alpha6 and alphav in JHU-022-SCC and UM-SCC-1 cell lines.

TABLE I.
Summary of Whole Cell Expression Levels of Different Integrin Subunits as Determined by Western Analysis.*

Integrin Subunit	Cell Type	
	SCC-1	022
Alpha2	+++	++
Alpha3	++	++
Alpha6	++	++++
AlphaV	++	+++
Beta1	++++	+++
Beta4	++	+++

*Relative expression of each integrin subunit is shown by + or − as determined by Western blot analysis. This is a semiquantitative measure of only whole cell integrin expression.

†Semiquantitative measurement of the whole cell integrin expression. Visual assessment of signal intensity after enhanced chemo-luminescence detection and autoradiography.

laminin 10 and laminin 11. Differences in adhesion to fibronectin were evident, with the UM-SCC-1 cells exhibiting greater attachment. The integrin alphaVbeta6 plays an important role in fibronectin attachment.[25] Flow cytometry analysis of alphaVbeta6 demonstrated an approximate doubling of this particular integrin's levels in the UM-SCC-1 cell line compared with JHU-022-SCC. An increased alphaVbeta6 could explain the greater affinity of the UM-SCC-1 cells for fibronectin.

Decreased adhesion of both cell lines to collagen IV and fibronectin in the presence of blocking antibodies to the alpha3 and alpha5 integrin subunits, respectively, suggests that these subunits are also important for both collagen IV and fibronectin adhesion. Blocking antibodies selective for the beta1 subunit demonstrated that this receptor subunit also plays a major role in collagen IV and fibronectin adhesion.

Interestingly, laminin 10 and laminin 11 adhesion by both cell lines remained unaffected by inhibiting function of the classic laminin binding subunits alpha3, alpha6, beta1 or beta4. These findings suggest as yet unrecognized mechanisms for binding to this substrate.

An interesting finding was the UM-SCC 1 cell line expression of the alpha2, alpha3, and alpha5 subunits, which was twice that of the JHU-022-SCC cell line by FACS analysis. This quantitative difference probably represents a cell surface phenomenon only for the alpha3 subunit because Western blot analysis suggested equal total cell levels of this integrin component. Nevertheless, the elevated surface levels of the alpha2 and alpha3 receptors (by FACS) correspond to the increased motility of the UM-SCC-1 cell line on laminin 5, probably through the use of the alpha3beta1 or alpha2beta1 receptor, or both.

Summary

The UM-SCC-1 and JHU-022-SCC head and neck squamous cell carcinoma cell lines have different invasive, migratory, and adhesive capabilities that may be explained in part by their patterns of cell surface receptors. Integrin expression and function may predict tumor behavior. In these studies, greater expression of the alpha6 and beta4 subunits and, presumably, the alpha6beta4 receptor was associated with a less invasive squamous cell carcinoma phenotype. Tumor migration along laminin 5 was seen with the receptors alpha3beta1 and alpha2beta1. An elevated level of the alphaVbeta6 receptor was associated with greater affinity for fibronectin. Further studies are needed to assess a wider variety of squamous carcinomas, their behavior, and their receptors.

CONCLUSION

A tumor's ability to spread along different matrix components may depend on a shift in the balance between stabilizing and motility forces. Although many cell types interact with collagen and other ECM components, similar associations between increased invasiveness of tumor fronts and along neural structures have only been demonstrated for the laminin 5substrate. It is likely that the laminin 5 motility receptors play a major role in the cell's ability to invade along this substrate in vitro and possibly in vivo. As an alternative, hemidesmosome formation between cells would tend to promote a nonmigratory phenotype. The alpha6beta4 receptor may act to limit invasion and migration even in the presence of laminin 5 and the appropriate migration receptors.

Differential integrin expression and interactive capabilities of tumors with the ECM may in part explain the different invasive properties. Future clinical application of this information may include targeting these interactions in an attempt to limit tumor spread or promote tumor anchorage and stability.

TABLE II.
Quantitative Analysis of Cell Surface Expression of Different Integrin Subunits as Determined by FACS.*

Cell Line	Integrin Subunit†									
	$\alpha 2$	$\alpha 3$	$\alpha 5$	$\alpha 6$	αV	B1	B3	B4	αVB6	Control
SCC-1	352	327	17.2	273.8	58.3	378.6	5.1	254.8	35.2	(3.9)
022	133	220	11.1	604.3	83.5	283.9	6.5	421.7	19.1	(2.6)
Factor‡	~0.5×	~0.5×	~0.5×	~2×	ND	ND	ND	~2×	~0.5×	(ND)

*Flow cytometry, determined cell surface expression levels of integrin subunits *$\alpha 2$, $\alpha 3$, $\alpha 6$, αV, B1, B3, B4, αVB6* in the JHU 022 SCC and UM SCC1 cell lines. Numerically represented (arbitrary units) in each row is the peak area under the curve obtained by FACS analysis. The control group contained cells that had not been exposed to anti-integrin antibodies.

†Numbers represent the flow cytometry peak area under the curves (arbitrary units).

‡Approximate multiple of JHU-022-SCC subunit expression relative to UM-SCC-1.

TABLE III.
Gross and Histologic Features From Tumors Grown in Athymic Mice From the UM-SCC-1 Cell Line versus the JHU-022-SCC Cell Lines.*

	Cell Line	
	SCC-1	022
Mice with tumor/total mice	8/8	7/7
Average tumor size	111 ± 31 cm^2†	107 ± 53 cm^2
Vascular invasion‡	++	++
Lymphatic invasion	+++	++
Neural invasion	+	−

*Top left photo demonstrates a section of nerve invaded by tumor derived from the UM-SCC-1 cell line. Bottom left photo demonstrates lymphatic invasion present in tumor specimens derived from both cell lines.
†Tumor surface area measured at day 30 using calipers.
‡Vascular, lymphatic and neural invasion were assessed by an independent pathologist.
§Representative pathologic slides demonstrating neural (upper) and lymphatic invasion (lower).

BIBLIOGRAPHY

1. Fagan J, Collins B, Barnes L, D'Amico F, Myers E, Johnson J. Perineural invasion in squamous cell carcinoma of the head and neck *Arch Otolaryngol Head Neck Surg* 1998;124: 637–640.
2. Hynes RO. Integrins: versatility, modulation, and signaling in cell adhesion. *Cell* 1992;69:11–25.
3. Mizejewski G. Role of integrins in cancer: survey of expression patterns. *Proc Soc Exp Biol Med* 1999;222:124–138.
4. Kanemoto T, Reich R, Royce L, et al. Identification of an amino acid sequence from the laminin A chain that stimulates metastasis and collagen IVase IV production. *Proc Natl Acad Sci U S A* 1990;87:2279–2283.
5. Yamamura K, Kibbey MC, Kleinman HK. Melanoma cells selected for adhesion to laminin peptides have different malignant properties. *Cancer Res* 1993;53:423–428.
6. Bagutti C, Speight PM, Watt FM. Comparison of integrin, cadherin, and catenin expression in squamous cell carcinomas of the oral cavity. *J Pathol* 1998;186:8–16.
7. Giancotti FG, Ruoslahti E. Integrin signaling. *Science* 1999; 285:1028–1032.
8. Pilewski J, Latoche JD, Arcasoy S, Albelda S. Expression of integrin cell adhesion receptors during human airway epithelial repair *in vivo*. *Am J Physiol* 1997;273:L256–263.
9. Smythe WR, LeBel E, Bavaria JE, Kaiser LR, Albelda SM. Integrin expression in non-small cell carcinoma of the lung. *Cancer Metastasis Rev* 1995;14:229–239.
10. Simon C, Nemechek A, Boyd D, et al. An orthotopic floor-of-mouth cancer model allows quantification of tumor invasion. *Laryngoscope* 1998;108:1686–1691.
11. Ziober B, Silverman S, Kramer R. Adhesive mechanisms regulating invasion and metastasis in oral cancer. *Crit Rev Oral Biol* 2001;12:499–510.
12. Zhang K, Kim JP, Woodley DT, Waleh NS, Chen YQ, Kramer

RH. Restricted expression and function of laminin1-binding integrins in normal and malignant oral mucosal keratinocytes. *Cell Adhes Commun* 1996;4:159–174.
13. Pyke C, Salo S, Ralfkiar E, Ramer J, Dane K, Tryggvason K. Laminin-5 is a marker of invading cancer cells in some carcinomas and is co-expressed with the receptor for urokinase plasminogen activator in budding cancer cells in colon adenocarcinomas. *Cancer Res* 1995;55:4132–4139.
14. Salo T, Autio-Harmainen H, Oikarinen A, Salo S, Tryggvason K, Salo T. Altered distribution and synthesis of laminin-5 (kalinin) in oral lichen planus, epithelial dysplasias, and squamous cell carcinomas. *Br J Dermatol* 1997;136: 331–336.
15. Ono Y, Nakanishi Y, Ino Y, et al. Clinicopathologic significance of lamini-5 gamma2 chain expression in squamous cell carcinoma of the tongue: immunohistochemical analysis of 67 lesions. *Cancer* 1999;85:2315–2321.
16. Anderson T, Feldman M, Weber R, Ziober A, Ziober B. Tumor deposition of laminin-5 and the relationship with perineural invasion. *Laryngoscope* 2001;111:2140–2143.
17. Carter WG, Kuar P, Gil SG, Gahr PJ, Wayner EA. Distinct functions for integrins [alpha3beta1] in focal adhesions and [alpha6beta4]/bullus antigen in a new stable anchoring contact (SAC) of keratinocytes: relation to hemidesmosomes. *J Cell Biol* 1990;111:3141–3154.
18. DiPersio CM, Hodivala-Dilke KM, Jaenisch R, Kreidberg JA, Hynes RO. Alpha3beta1 integrin is required for normal development of the epidermal basement membrane. *J Cell Biol* 1997;137:129–742.
19. Yauch RL, Berditchevski F, Harler MB, Reichner J, Hemler ME. Highly stoichiometric, stable, and specific association of integrin alpha3beta1 with CD151 provides a major link to phosphatidylinositol 4-kinase, and may regulate cell migration. *Mol Biol Cell* 1998;9:2751–2765.
20. Decline F, Rousselle P. Keratinocyte migration requires [alpha2beta1] integrin-mediated interaction with the laminin 5 alpha2 chain. *J Cell Sci* 2001;114:811–823.
21. Alt A, Ohba M, Li L, et al. Protein kinase Cdelta-mediated phosphorylation of alpha6beta4 is associated with reduced integrin localization to the hemidesmosome and decreased keratinocyte attachment. *Cancer Res* 2001;61:4591–4598.
22. Rabinovitz I, Toker A, Mercurio AM. Protein kinase C-dependent mobilization of the alpha6beta4 integrin from hemidesmosomes and its association with actin rich cell protrusions drive the chemotactic migration of carcinoma cells. *J Cell Biol* 1999;146:1147–1160.
23. Lotus S, Geuijen CAW, Oomne LCJM, Calfat J, Janssen H, Sonnenberg A. The tetraspan molecule CD 151, a novel constituent of hemidesmosomes, associates with the integrin alpha6beta4 and may regulate the spatial organization of hemidesmosomes. *J Cell Biol* 2000;149:969–982.
24. Liebert M, Washington R, Wedemeyer G, Carey TE, Grossman HB. Loss of co-localization of alpha6 beta4 integrin and collagen VII in bladder cancer. *Am J Pathol* 1994;144:787–795.
25. Weinacker A, Chen A, Agrez M, et al. Role of the integrin alphaV beta 6 in cell attachment to fibronectin: heterologous expression of intact and secreted forms of the receptor. *J Biol Chem* 1994;269:6940–6948.