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Characterization of integrin subunits, cellular adhesion and tumorgenicity of four human prostate cell lines

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Abstract. Cellular adhesion to extracellular matrix proteins via integrin molecules is a major factor in the process of invasion and metastasis of human tumor cells. Four human prostate cell lines were characterized according to the presence and quantity of integrin subunits, the ability of the cells to attach to extracellular substrates and the capacity of the cells to form tumors in severe combined immunodeficient (SCID) mice. All four human prostate cell lines expressed three to five integrins on their cell surfaces. The DU145, PC3 and 431P cells expressed primarily α_3, α_5, and α_6 integrin at similar levels. These cell lines expressed the subunits β_1, β_3, and β_4 with β_1 predominant. The DU145 cells preferred attachment to fibronectin, followed by laminin and vitronectin. Approximately 50%–60% of the binding of DU145 cells to fibronectin and laminin was dependent on the function of $\alpha_5\beta_1$ and α_6 respectively. The cell line LNCaP differed in its low expression of the α_3 subunit, 95% of cellular adhesion to fobronectin and laminin being integrin-dependent and its inability to attach to vitronectin, in spite of surface expression of $\alpha_v\beta_3$. All the cell lines except for LNCaP readily formed tumors within SCID mice and the expression of α_3, α_6, β_1 and β_4 integrin subunits was preserved in the resulting tumor tissue. The altered adhesion properties of the LNCaP cells may explain their altered tumorigenicity.

Key words: Integrins – Cell adhesion – Human prostate cell lines – Metastasis

Introduction

The process of tumor formation, invasion and metastasis is an intricate one that involves cellular attachment to the extracellular matrix, local proteolysis of the basement membrane and migration through the stroma to gain access to the circulation for the establishment of a metastatic colony (Liotta and Kohn 1990). Within this cascade, aberrant adhesion is known to be involved in the metastatic process in human cancers (Liotta and Kohn 1990); migratory cells display, in part, a loss of cellular adhesiveness (Ruoslahti and Giancotti 1989; Burridge 1986). The process of cellular adhesion to extracellular matrix proteins (collagen, fibronectin, laminin, and vitronectin) as well as cell-cell adhesion is mediated in part by transmembrane proteins that have been termed integrins. A functional integrin is a heterodimer composed of an α and β subunit non-covalently linked (Hynes 1992). Both subunits are integral membrane proteins with a large extracellular domain, a transmembrane domain, and a cytoplasmic domain (Albelda and Buck 1990). The β_1 subunits communicate with the actin cytoskeleton via talin, vinculin, fibulin and α-actinin (Albelda and Buck 1990; Hogervorst et al. 1990; Burridge et al. 1988).

An alteration of integrin composition has been observed to correlate with metastatic potential. For example, an investigation of integrin expression in histological sections of human malignant melanoma suggests that expression of β_3 was most prevalent on melanoma cells in the vertical growth phase and that β_3 expression correlates with increased metastatic potential (Albelda et al. 1990). In addition, alternative integrin units have been found on melanoma cells (Kramer et al. 1991). These changes in integrin expression on cancer cells suggested a selective growth advantage, the ability to adhere and invade the basement membrane in conjunction with its degradation and the ability to metastasize to distant sites (Feldman et al. 1991; Constantini et al. 1990). More recent studies have shown that overexpression of $\alpha_2\beta_1$ in rhabdomyosarcoma cells in vitro causes an increase in formation of metastatic tumors after injection of the cells into nude mice (Bosco et al. 1991). An increase in expression of $\alpha_6\beta_1$, a laminin receptor, has been correlated with an increase in basement membrane invasion by chemically transformed human HOS cells (Dedhar and Saulnier 1990). There is also evidence that glycosylation of β_1 may also affect the invasiveness of cells (Dedhar and Saulnier 1990). However, in

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Abbreviations: SCID, severe combined immunodeficiency; FITC, fluorescein isothiocyanate; FACS, fluorescence-activated cell sorting; PBS, phosphate-buffered saline; FBS, fetal bovine serum

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other studies the increase in expression of $\alpha_5\beta_1$, the fibronectin receptor, correlates with an increased adhesiveness to fibronectin and less aggressive behavior in transformed rodent cells (Plantefaber and Hynes 1989).

In this study, we characterized the cell-surface integrins expressed and the adhesive properties to specific substrates of human prostate cell lines isolated from human metastatic tumors. The tumorigenicity of these cells was determined by intraperitoneal injection into mice containing the severe combined immunodeficiency (SCID) mutation. The SCID mutation results in mice that are deficient in functional T and B cells and are deficient in all major classes of immunoglobulins (Ansell and Bancroft 1989). The SCID mice can support the growth of allogeneic and xenogenic tumors (Bosma and Carroll 1991).

Materials and methods

Cell lines and antibodies. DU145 (ATCC HTB 81) originally was isolated from a lesion of the brain in a patient with metastatic carcinoma of the prostate (Stone et al. 1978). The PC3 (ATCC CRL 1435) cell line was initiated from a grade-IV prostatic adenocarcinoma (Kaighn et al. 1979). The LNCaP (ATCC CRL 1740) cell line was isolated from a needle aspiration biopsy of a supraclavicular lymph node metastatic prostate adenocarcinoma (Horoszewicz et al. 1983). All cell lines were obtained from the American Type Tissue Culture Collection, Rockville, Maryland. 431P is a variant of the PC3 cell line (Dr. D. H. Shevrin, University of Illinois, Chicago, Ill.) that was obtained by serially passaging PC3 in nude mice (Shevrin et al. 1986, 1988). Cells were maintained in Iscove's modified Dulbecco's medium, 10% fetal bovine serum (FBS), 1% L-glutamine (2300 mM, Gibco), and 1% penicillin/streptomycin (10 000 U/ml and 10 000 µg/ml respectively).

The antibodies to integrin, subunits included monoclonal antibodies to α_2 (P1E6, Telios, San Diego, Calif.) (Wayner et al. 1988), α_3 (P1B5, Telios) (Wayner et al. 1988), α_4 (P4G9, Telios) (Wayner et al. 1989), α_5 (P1D6, Telios) (Wayner et al. 1988), α_6 (GoH3) a gift from Dr. A. Sonnenberg (Sonnenberg et al. 1987), α_v (VNR 147, Telios) (Freed et al. 1989; Vogel et al. 1990), β_1 (A-1A5) a gift from Dr. M. E. Hemler (Hemler et al. 1983), ß$_2$ (P4H9, Telios), β_3 (4B12) (Genentech Inc., South San Francisco, Calif.), β_4 (3E1, Telios) (Hessle et al. 1984) and a polyclonal antibody to $\alpha_v\beta_3$ (Calbiochem, La Jolla, Calif.). The anti-keratin antibody, designated 10, 11 (a gift from Dr. Robert Cardiff) is specific for human keratins 8 and 18 (Chan et al. 1986). Secondary antibodies used were fluorescein-isothiocyanate(FITC)-conjugated goat anti-(mouse IgG), FITC-conjugated goat anti-(rabbit IgG) and FITC-conjugated goat anti-(rat IgG) (Cappel, Durham, N.C.). Primary antibodies were used at a dilution of 1 : 100 and secondary antibodies were used at a dilution of 1 : 500 for immunofluorescence and at a 1 : 1000 dilution for FACS analysis.

Indirect immunofluorescence microscopy. Cell lines were grown on coverslips in 35-mm petri dishes and allowed to proceed through one or two population doublings. Coverslips were washed three times in phosphate-buffered saline (PBS: 150 mM NaCl, 700 µM KH_2PO_4, 4 mM K_2HPO_4), fixed in cold acetone for 5 min and allowed to dry in air. Cells were rehydrated with 1% bovine serum albumin (BSA) in PBS for 5 min and the coverslips were incubated at room temperature with 50 µl of a 1 : 100 dilution of the primary antibody for 30 min. Coverslips were washed three times with PBS and then flooded with the appropriate secondary antibody (for 30 min at room temperature). Coverslips were washed three times with PBS and then mounted onto slides with 10% Gelvatol (Monsanto, St. Louis, Mo.) in a PBS/glycerol base.

Tumors were excized from the SCID mice and snap-frozen in liquid-nitrogen-cooled isopentane. Serial 3- to 5-µm sections were put on slides and acetone-fixed for 5 min then allowed to dry in air. Sections were rehydrated in PBS for 5 min and excess PBS was drained from the slide. The slides were incubated with 50 µl of a 1 : 100 dilution of integrin monoclonal antibodies or monoclonal antibody (10, 11) specific for human cytokeratins 8 and 18 for 30 min at room temperature in a humidified chamber. The slide was flooded with PBS and allowed to rinse for an additional 5 min, then the excess was drained from the slide. The slides were then flooded with the appropriate FITC-conjugated or rhodamine-conjugated secondary antibody for 30 min at room temperature in a humidified chamber. Slides were again flooded with PBS and allowed to rinse for 5 min in a Coplin jar. Coverslips were mounted on slides using 10% Gelvatol and stored at 4° C.

Fluorescence-activated cell sorting (FACS). Cells were removed from the tissue-culture dish with a rubber policeman and washed twice in cold PBS. Cells suspensions were kept on ice. Cell pellets were resuspended in 300 µl of a 1 : 100 dilution of primary monoclonal or polyclonal antibody and incubated for 30 min. Cell pellets were washed three times in PBS+1% FBS+10 mM sodium azide, resuspended in 500 µl FITC-conjugated secondary antibody and incubated in the dark for 30 min. Cells were fixed in 1% formaldehyde at a final concentration of 1×10^6 cells/ml for FACS analysis. At least 5000 cells were analyzed on a Becton Dickinson FACScan system. Data points were collected using Consort 30 software and composite histograms were analyzed using Kolomogorov-Smirnorv statistics. The critical value, D_{crit}=0.0326, was calculated for the 99% confidence level using the table of critical values and confidence levels for a Kolomogorov-Smirnorv two-sample test (Young 1977).

Immunoprecipitation of cell-surface integrins. The proteins on the cell surface were biotinylated using the procedure of Isberg and Leong (1990). The cells were washed with a buffer containing 50 mM TRIS/HCl pH 7, 0.15 M NaCl, 1 m*M* $CaCl_2$, 1 m*M* $MgCl_2$ and lysed using the wash buffer containing 1% NP40, 2 m*M* phenylmethylsulfonyl fluoride, 1 µg/ml leupeptin and 1 µg/ml aprotinin with brief sonication. The integrin subunits were retrieved from the resulting mixture of surface-labeled proteins by immunoprecipitation using integrin-subunit-specific monoclonal antibodies, incubated for 18 h at 4° C. The antibodies used were obtained from the following sources: α_3 (P1B5, Telios), α_5 (P1D6, Telios), α_6 (GoH3, Accurate Chemical and Scientific Corp., N.Y., and $\alpha_v\beta_3$ (polyclonal, Calbiochem). The immunocomplexes were retrieved using protein-A-Sepharose or a mixture of protein-G-Sepharose and anti-(rat Ig)-agarose and analyzed using non-denaturing 7.5% polyacrylamide gel electrophoresis. The proteins were transferred to nitrocellulose and visualized by chemiluminescence using peroxidase/streptavidin and the ECL Western blotting detection system kit (Amersham).

Substrate attachment and inhibition of attachment. The protocol used was a modification of a procedure by Cheresh at al. (Cheresh et al. 1989). Individual wells of a 96-well Immulon microtiter plate (Dyna Tech Laboratories Inc., Chantilly, Va.) were coated with human vitronectin, human fibronectin (both from Telios Pharm. Inc.) or human laminin (Gibco Corp.) at a protein concentration of 10 µg/ml. Triplicate wells were coated for each condition with 100 µl protein solution, sealed with Parafilm and allowed to incubate overnight. Before plating cells, wells were rinsed with cold PBS.

Cells were metabolically labeled with 30 µCi/ml [^{35}S]methionine (NEN, Expre^{35}S^{35}S, methionine/cysteine labeling mix) in 3 ml McCoys medium deficient in methionine for 3 h. Cells were removed from the flask by trypsinization and the cell suspensions were rinsed three times in cold PBS. [^{35}S]Methionine (cpm)/100µl was determined and approximately 20 000 cells (1×10^5 cpm) were added to each well. Cells were plated onto substrate-coated wells in triplicate and allowed to attach for 1 h.

For inhibition of attachment, 300 µl cell suspension was divided into aliquots in 13×75-mm glass tubes on ice and GoH3 or P1D6 antibodies were added at a 1 : 100 dilution. All samples were incubated for 15 min at 4° C. After incubation, 100 µl cells was plated into one of three coated wells for each condition. The time allowed for attachment was 1 h in a 5% CO_2 humidified 37° C incubator. After attachment, wells were washed three times with PBS and cells that remained attached were solubilized with 200 µl 2% sodium dodecyl sulfate in PBS. Radioactivity (cpm) that remained was determined for each well. Tissue-culture 96-well plates were used as the positive control for these experiments since the cells were known to attach to the treated plastic.

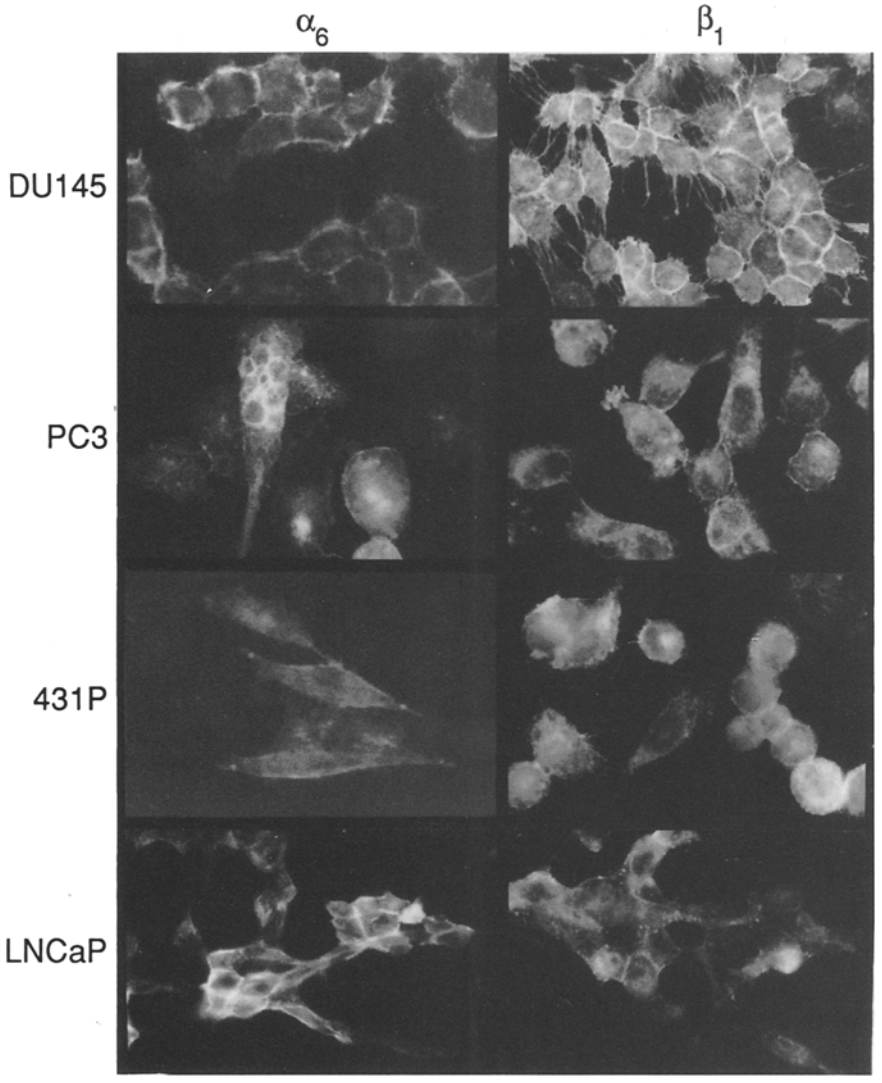

Fig. 1. Indirect immunofluorescence detection of the integrin subunits α_6 and β_1 on human prostate cell lines. Monoclonal antibodies were used to detect the integrin subunits on DU145, PC3, 431P and LNCaP cells. The presence of the antigen-antibody complex was detected by fluorescein-isothiocyanate (FITC)-conjugated goat anti-(mouse IgG) or anti-(rat IgG). Manification ×400

Cells were plated onto untreated wells of the Immulon 96-well plate as negative controls, as these cells will not attach to untreated plastic.

Experimental tumorigenicity assay. At least 1×10^7 viable tumor cells were injected intraperitoneally into SCID mice and animals were submitted to autopsy approximately 25 days after injection. Tumor cell viability was determined using the trypan blue dye exclusion test. The animals were screened for immunodeficiency by determining the concentration of IgG present in the serum using an enzyme-linked immunosorbent assay (ELISA). Male and female mice with IgG less than 1.0 ng/dl were used.

Results

Indirect immunofluorescence microscopy

Indirect immunofluorescence microscopy was used to detect the presence and distribution of integrin subunits within the human prostate cell lines. Figure 1 shows a representative example of the indirect immunofluorescence microscopy of all four cell lines using monoclonal antibodies to α_6 and β_1 subunits. Although the cell lines express surface α_6, the distribution of the integrin is different. For example, 431P cells show a diffuse cytoplasmic membrane pattern of α_6 distribution whereas DU 145, PC3 and LnCAP cells contain α_6 in focal regions on the cell surface. The DU145 and LNCaP cells contain α_6 and β_1 in a similar distribution. Within the cell line populations, variability was noted in the apparent amount and distribution of integrin present on the individual cell surfaces.

Fluorescence-activated cell sorter (FACS) analysis

Figure 2 shows FACS histograms for each α subunit antibody on the prostate cell lines. Composite histograms were produced comparing the negative-control histogram, cells incubated with only the appropriate FITC-conjugated second antibody (solid line), to the same cells first incubated with monoclonal antibodies for specific α integrin subunits (dotted line).

The DU145 cell line shows a strong positive shift in fluorescence intensity with monoclonal antibodies (mAb) to α_3, α_5, α_6, and α_v and a weak positive shift with the α_2 mAb. The

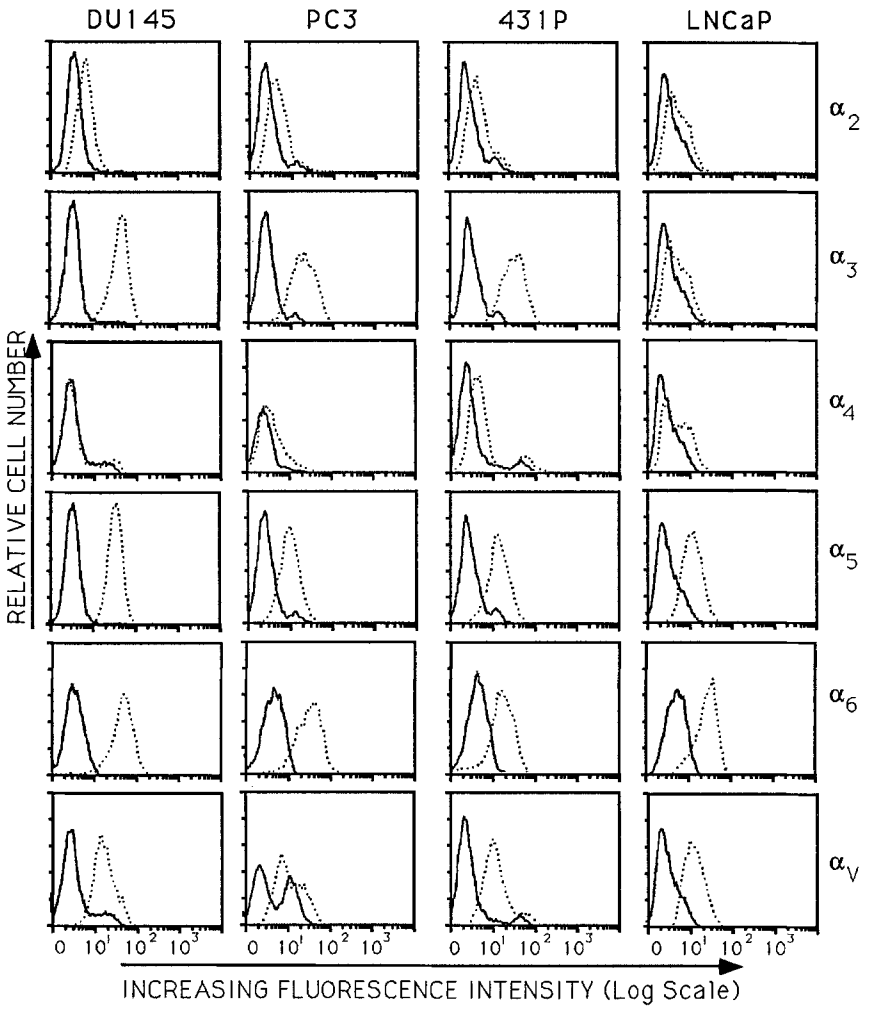

Fig. 2. Fluorescence-activated cell sorter (FACS) analysis of the integrin subunits α_2, α_3, α_4, α_5, α_6, and α_v on four human prostate carcinoma cell lines. The cells were reacted with each monoclonal antibody. The composite histograms shown for each α subunit contain the secondary-only control histogram (—) and the histogram obtained after incubation with a monoclonal antibody to an α subunit (···). *Column 1,* DU145; *column 2,* PC3; *column 3,* 431P; *column 4,* LNCaP. The α subunits represented in each *row* are indicated to the *right* of the figure

Table 1. Fluorescence-activated cell sorter (FACS) analysis of α and β subunits on human prostate cell lines [a]

Cell line	Integrin subunits (median channel of peak fluorescence)									
	α_2	α_3	α_4	α_5	α_6	α_v	β_1	β_2	β_3	β_4
DU145	7.03	51.26	2.47 *	38.40	46.00	15.01	13.42	3.81 *	9.03	17.98
PC3	5.08	22.34	2.75 *	9.73	46.00	8.42	7.29	2.30 *	12.53	15.57
431P	4.90	26.7	4.09	12.53	12.99	11.25	5.66	2.65 *	13.47	2.85
LNCaP	3.54	3.06	3.29	12.53	49.44	10.85	8.42	3.81	2.75	3.67

[a] Critical D value is 0.0326 for the 99% confidence level
* D values not significant

PC3 cell line shows a strong positive shift in fluorescence intensity with α_3 and α_6 mAb, and a moderate shift with α_2, α_5, α_v. The 431P cell line, which has been characterized as a highly metastatic derivative of the PC3 cell line (Dr. Shevrin, University of Illinois), produced a moderate reaction with α_3, α_5, α_6 and α_v. All three of these cell lines showed no positive reaction with the α_4 mAb. The LNCaP cell line was different in that it reacted weakly with α_2 and α_3 mAb. Table 1 shows the median channel of peak fluorescence from the antibody-treated histogram (dotted histograms from Fig. 2) for all four human cell lines using the monoclonal antibodies to the α subunits. The relative quantities of each α subunit on each cell line can be compared.

Figure 3 shows FACS histograms of the human prostate cell lines using monoclonal antibodies to the β subunits. The DU145, PC3 and 431P cell lines show a positive shift in fluorescence intensity with mAb to β_1, β_3, and β_4. LNCaP cells show very minor peak shifts with β_1, β_2, β_3, and β_4. Table 1 shows the median channel of peak fluorescence from the antibody-treated histogram (dotted histograms from Fig. 3) for all four human cell lines using the monoclonal antibodies to the β subunits. The relative quantities of each β subunit can be compared.

The Kolomogorov-Smirnorv test (Young 1977) was used to test whether the negative-control histogram and the histogram produced by incubation of cells with the different

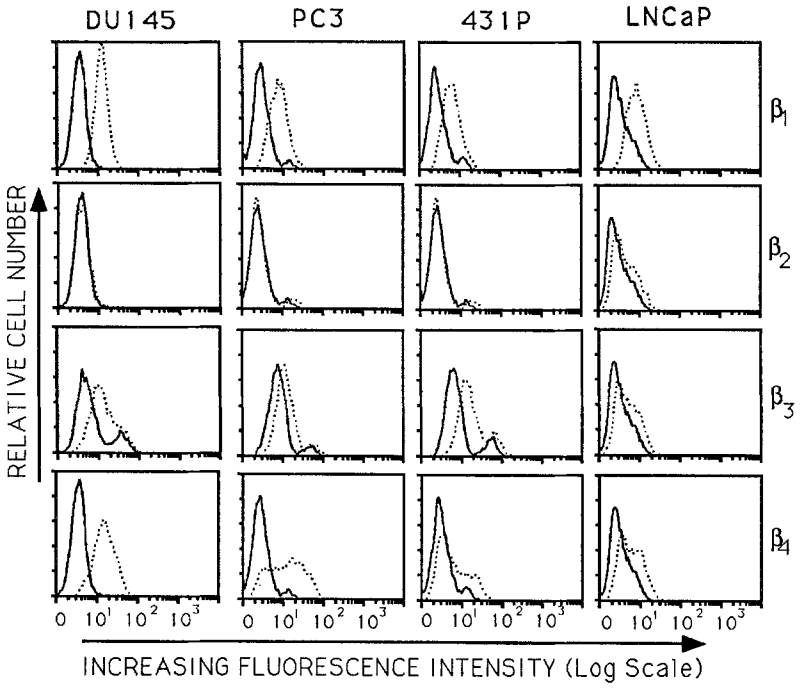

Fig. 3. Fluorescence-activated cell sorter analysis using monoclonal antibodies to β subunits, β_1, β_2, β_3, and β_4. The composite histograms are shown for all cell lines with all four β subunit antibodies. Composite histograms contain the secondary-only control histogram (—) and the histogram obtained after incubation with a monoclonal antibody to a β subunit (···). *Column 1,* DU415; *column 2,* PC3; *column 3,* 431P; *column 4,* LNCaP. The β subunits represented in each *row* are indicated to the *right* of the figure

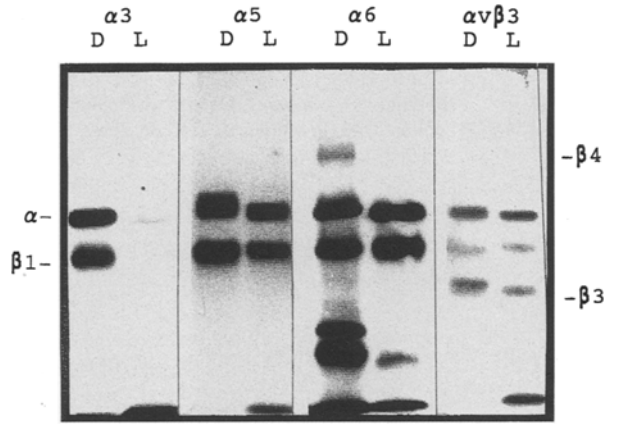

Fig. 4. Immunoprecipitation of the integrin subunits. The surface-exposed integrins on DU145 (*D*) and LNCaP (*L*) cells were labeled using biotin and immunoprecipitated using monoclonal antibodies specific for α_3, α_5, or α_6 and polyclonal antibody specific for $\alpha_v\beta_3$. The resulting proteins were analyzed using non-denaturing 7.5% polyacrylamide gel electrophoresis, transferred onto nitrocellulose and visualized by chemiluminescence

monoclonal antibodies to the integrin subunits were significantly different. The composite histograms compared for α and β subunits (Figs. 2 and 3) were subjected to the Kolomogorov-Smirnorv two-sample test. The histograms produced by the monoclonal antibodies that were not significant are indicated in Table 1 by asterisks.

Immunoprecipitation of cell-surface integrins

Figure 4 shows the resulting immunoprecipitation of the integrin units $\alpha_{3,5,6}$ and $\alpha_v\beta_3$ from DU145 and LNCaP cells. Both cell lines contain the $\alpha_5\beta_1$, $\alpha_6\beta_1$, and $\alpha_v\beta_3$ integrins on the cell surface. These data are consistent with the data obtained by FACS analysis shown in Fig. 2 and Table 1. In addition we consistently observed on the surface of the DU145 cells, using the anti-α_6 antibody, the β_4 integrin and immunoprecipitating proteins of 70 kDa and 50 kDa. The identity of these proteins is unknown. Using a polyclonal antibody specific for $\alpha_v\beta_3$, we detected on both cell lines an integrin unit of apparently 100 kDa molecular mass, which is consistent with the surface expression of the β_5 subunit of the $\alpha_v\beta_5$ heterodimer as reported by others (Smith et al. 1990; Wayner et al. 1991). The LNCaP cell line did not express $\alpha_3\beta_1$ on the cell surface as compared to the DU145 cell line, again confirming the FACS analysis. The α integrin subunits from the LNCaP line migrated faster than those obtained from the DU145 cell line, suggesting that the LNCaP integrins may be defective in a posttranslational modification.

Substrate attachment and inhibition of attachment

Experimental substrate attachment was used to test the function of $\alpha_5\beta_1$ (fibronectin), $\alpha_6\beta_1$ or $\alpha_6\beta_4$ (laminin) and $\alpha_v\beta_3$ or $\alpha_v\beta_5$ (vitronectin) (Fig. 5). Both the DU145 and LNCaP cell lines attached to human fibronectin and human laminin. Approximately 50%–60% of the attachment of DU145 cells to fibronectin and laminin is inhibited by the anti-$\alpha_5\beta_1$ antibody (P1D6) and anti-α_6 antibody (GoH3) respectively. The remaining attachment to fibronectin and laminin may occur through $\alpha_3\beta_1$ since this integrin is observed by FACS analysis (Table 1) or attachment may be integrin-independent. Approximately 97% of the attachment of LNCaP cells to fibronectin and laminin is inhibited by the anti-$\alpha_5\beta_1$ antibody and the anti-α_6 antibody respectively. In contrast to the DU145 cells, the LNCaP cells showed no attachment to vitronectin within the 1-h incubation period. This is an unexpected result since the cells contain surface-expressed $\alpha_v\beta_3$ as detected by FACS analysis (Table 1) and immunoprecipitation (Fig. 4).

Experimental tumorigenicity assay

The DU145 cells formed multiple microscopic tumors in the SCID mice with occasional bloody ascites (Table 2). The tu-

mors formed from this cell line were small and found to be noninvasive. PC3 and 431P cells formed grossly evident peritoneal tumors. Both PC3 and 431P tumors grew aggressively and upon histological analysis were shown to be invading the muscle tissue of the diaphram. In no case were metastases found outside the peritoneal cavity. Both of these cell lines were reduced in the α_5 subunit compared to DU145

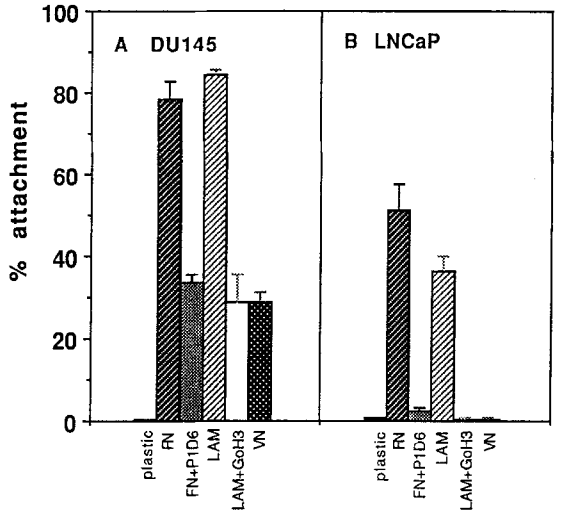

Fig. 5 A, B. Experimental substrate-attachment assay. The percentage of attachment is defined as 100× (amount of cells that attach)/(cells applied to the well). The standard error of the mean is shown for each substrate. DU145 cells (**A**) or LNCaP cells (**B**) were tested for attachment to uncoated plastic (*plastic*), fibronectin (*FN*), laminin (*LAM*) or vitronectin (*VN*). The inhibition of attachment was tested by incubating the cells with anti-$\alpha_5\beta_1$ (PD16) or anti-α_6 (GoH3) antibody prior to testing attachment

Table 2. Human prostate cell line tumorigenicity and integrin content in murine tumors [a]

Cell lines	SCID mouse tumours	Integrin subunits
DU145	Multiple, microscopic	$\alpha_3\,\beta_1, \beta_4$
PC3	Gross, peritoneal, invasive	$\alpha_6, \beta_1, \beta_4$
431P	Gross, peritoneal, invasive	$\alpha_3\,\alpha_6, \beta_1$
LNCaP	None	

[a] Four mice were injected for each cell line. The presence of integrins α_{2-6} and β_{1-4} was tested within the tumors by indirect immunofluorescence microscopy. SCID, severe combined immunodeficient

cells (Fig. 2 and Table 1). The LNCaP cell line, as shown, up to 33 days after injection did not produce tumors.

Immunofluorescence microscopy was used to determine the integrin expression in the human tumor tissue from the SCID mice. Figure 6 shows that human tumor cells were growing within the SCID mouse. All resulting tumors were sectioned and serial sections were incubated with a human-specific keratin monoclonal antibody and an integrin subunit monoclonal antibody. This technique was used to verify that the tumor contained human tumor cells and to analyze the integrin expression in the resulting tumors. The integrin monoclonal antibodies used on the tumor sections revealed positive staining with α_3 on DU145 and 431P tumors and β_1 on all tumors (Table 2). The α_6 integrin was found primarily on the tumors formed by injection of the metastatic cell-line variants PC3 and 431P. Similar to the tissue-culture lines (Table 1), β_1 was positive on all human tumors.

Discussion

Integrins are thought to be involved in the process of invasion and metastasis either by inappropriate or altered integrin receptor expression (Ruoslahti and Giancotti 1989; Chelberg et al. 1989; Long et al. 1989; Sonnenberg et al. 1988). Little is known about integrin expression in human prostate cells and how this effects tumorigenicity. We have initially chosen a model system that includes four human prostatic cell lines isolated from different metastatic sites.

All four cell lines were found to express relatively similar amounts of α_2, α_4, α_6, α_v and β_1 consistently. The DU145 cells expressed the greatest amount of α_5 when compared to the other three cell lines. The DU145 cells formed microscopically sized tumors, which were not found to be invading. The PC3 and 431P cells, however, expressed less α_5 and were found to be invading surrounding tissues. Ruoslahti and Giancotti (1989) have suggested that expression of high levels of the $\alpha_5\beta_1$ integrin reduces cell migration. Our data are consistent with this observation.

Dedhar and Saulnier (1990) have demonstrated the role of $\alpha_6\beta_1$ in basement membrane invasion by using a monoclonal antibody against α_6 to inhibit invasion of chemically transformed cells through a reconstituted membrane. All three tumor-forming cell lines expressed similar levels of α_6 on the cell surfaces before injection into the SCID mice. After injection, the invasive tumors formed by the PC3 and 431P cells were found to express α_6 and β_1. The preservation of ex-

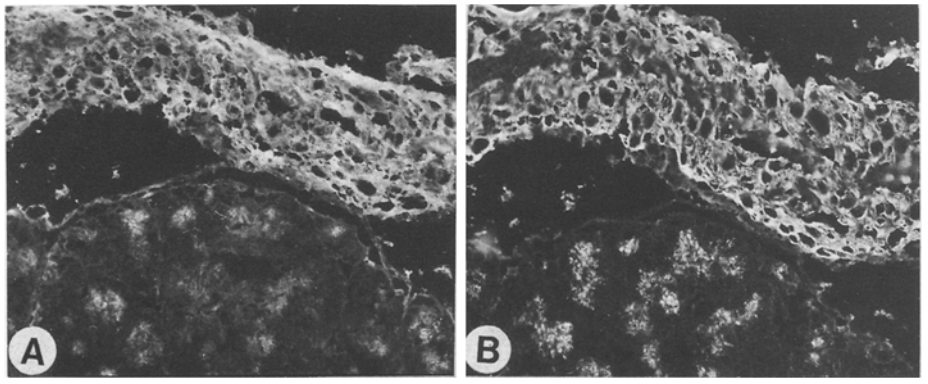

Fig. 6 A, B. Double immunofluorescence of cell line DU145 from a tumor section from a severe combined immunodeficient (SCID) mouse. **A** Distribution of β_1 integrin subunits on the human prostate cells. **B** Distribution of human cytokeratin using a monoclonal antibody designated 10, 11. The keratin-specific fluorescence verified human prostate cells in the tumor within the SCID mouse

pression within the tumors of this integrin combination may indicate a role for this heterodimer in tumor progression.

We also find it interesting that the DU145 cells, which were not invasive, formed microscopic tumors and expressed the greatest amount of β_4 (Fig. 3), compared to the other cell lines. The α_6 is known to complex with β_1 and β_4 and be a receptor for laminin (Sonnenberg et al. 1988; Lee et al. 1992); our data (Fig. 5) indicate the α_6 on these cells is present as both $\alpha_6\beta_1$ and $\alpha_6\beta_4$ complexes. In comparison, the invasive PC3 and 431P cell lines do not contain surface-expressed β_4 (Fig. 3), suggesting primarily $\alpha_6\beta_1$ complexes. The role of α_6 in tumor progression may depend upon its association with β_1.

The LNCaP cells show surface expression of $\alpha_v\beta_3$, as detected by immunofluorescence and immunoprecipitation but these cells do not functionally attach to vitronectin. This is a curious result since vitronectin is known to be the ligand for $\alpha_v\beta_3$ (Cheresh et al. 1989; Wayner et al. 1991). These data suggest the presence of inactive integrin subunits on the LNCaP cell surface, which may be related to the lower apparent molecular mass of the integrin subunits observed by immunoprecipitation (Fig. 4). The activation of integrin subunits has been previously described as a mechanism to regulate integrin function (Du et al. 1991).

The activation of integrin receptors may require cooperativity between receptors mediated by an extracellular ligand (Du et al. 1991; Bauer et al. 1992; La Flamme et al. 1992). The activation of the laminin receptors by the ligand may explain the observations that incubation of the LNCaP cells with extracellular matrix components prior to injection allows tumor formation (Pretlow et al. 1991). The extracellular matrix may play a role in prostate cancer growth and metastasis (Passaniti et al. 1992; Gleave et al. 1991, 1992; Chung 1991; Chung et al. 1991; Freeman et al. 1991) via the expression and activation of integrin heterodimers.

References

Albelda SM, Buck CA (1990) Integrins and other cell adhesion molecules. Integrin Review. FASEB J 4:2868–2880

Albelda SM, Mette SA, Elder AE, Stewart RM, Damjanovich L, Herlyn M, Buck CA (1990) Integrin distribution in malignant melanoma: association of the β_3 subunit with tumor progression. Cancer Res 50:6757–6764

Ansell JD, Bancroft GJ (1989) The biology of the SCID mutation. Immunol Today 10:322–325

Bauer JS, Schreiner CL, Giancotti FG, Ruoslahti E, Juliano RL (1992) Motility of fibronectin receptor-deficient cells on fibronectin and vitronectin: collaborative interactions among integrins. J Cell Biol 116:477–487

Bosco M, Chan C, Matsuura N, Takada Y, Zetter BR, Hemler ME (1991) In vitro and in vivo consequences of VLA-2 expression on rhabdomyosarcoma cells. Science 251:1600–1602

Bosma MJ, Carroll AM (1991) The SCID mouse mutant: definition, characterization and potential uses. Annu Rev Immunol 9:323–350

Burridge K (1986) Substrate adhesions in normal and transformed fibroblasts: organization and regulation of cytoskeletal, membrane and extracellular matrix components at focal contacts. Cancer Res 4:18–78

Burridge K, Fath K, Kelly T, Nuckolis B, Turner C (1988) Focal adhesions: transmembrane junctions between the extracellular matrix and the cytoskeleton. Annu Rev Cell Biol 4:487–525

Chan R, Rossito PV, Edwards BF, Cardiff RD (1986) Presence of proteolytically processed keratins in culture medium of MCF-7. Cancer Res 46:6353–6359

Chelberg MK, Tsilibrary EC, Hauser AR, McCarthy JB (1989) Type IV collagen-medicated melanoma cell adhesion and migration: involvement of multiple, distinct domains of the collagen molecule. Cancer Res 49:4796–4802

Cheresh DA, Smith JW, Cooper HM, Quaranta V (1989) A novel vitronectin receptor integrin ($\alpha_v\beta_x$) is responsible for distinct adhesive properties of carcinoma cells. Cell 57:59–69

Chung LWK (1991) Fibroblasts are critical determinants in prostatic cancer growth and dissemination. Cancer Metastasis Rev 10:263–274

Chung LWK, Gleave ME, Hsieh JT, Hong SJ, Zhau HE (1991) Reciprocal mesenchymal-epithelial interaction affecting prostate tumor growth and hormonal responsiveness. Cancer Surv 11:91–121

Costantini RM, Falcioni R, Battista P, Zupi G, Kennel SJ, Colasante A, Venturo I, Curcio CG, Sacchi A (1990) Integrin (α_6/β_4) expression in human lung cancer as monitored by specific monoclonal antibodies. Cancer Res 50:6107–6112

Dedhar S, Saulnier R (1990) Alterations in integrin receptor expression on chemically transformed human cells: specific enhancement of laminin and collagen receptor complexes. J Cell Biol 110:481–489

Du XP, Plow EF, III Frelinger AL, O'Toole TE, Loftus JC, Ginsberg MH (1991) Ligands "activate" integrin $\alpha_{11}b\beta_3$ (platelet GPIIb-IIIa). Cell 65:409–416

Feldman LE, Shin KC, Natale RB, Todd RF (1991) β_1 integrin expression on human small cell lung cancer cells. Cancer Res 51:1065–1070

Freed E, Gailit J, van der Geer P, Ruoslahti E, Hunter T (1989) A novel integrin ß subunit is associated with the vitronectin receptor a subunit (α_v) in a human osteosarcoma cell line and is a substrate for protein kinase C. EMBO J 8:2955–2965

Freeman MR, Song Y, Carson DD, Guthrie PD, Chung LWK (1991) Extracellular matrix and androgen receptor expression associated with spontaneous transformation of rat prostate fibroblasts. Cancer Res 51:1910–1916

Gleave M, Hsieh JT, Gao C, von Eschenbach AC, Chung LWK (1991) Acceleration of humanprostate cancer growth in vivo by factors produced by prostate and bone fibroblasts. Cancer Res 51:3753–3761

Gleave ME, Hsieh JT, von Eschenbach AC, Chung LWK (1992) Prostate and bone fibroblasts induce human prostate cancer growth in vivo: implications for bidirectional tumor-stromal cell interaction in prostate carcinoma growth and metastasis. J Urol 147:1151–1159

Hemler ME, Ware CF, Strominger JL (1983) Characterization of a novel differentiation antigen complex recognized by a monoclonal antibody (A-1A5): unique activation-specific molecular forms on stimulated T cells. J Immunol 131:334–340

Hessle H, Sakai L, Hollister D, Burgeson R, Engvall E (1984) Basement membrane diversity detected by monoclonal antibodies. Differentiation 26:49–54

Hogervorst F, Kuikman I, von dem Borne AEG, Sonnenberg A (1990) Cloning and sequence analysis of beta-4 cDNA: an integrin subunit that contains a unique 118 kd cytoplasmic domain. EMBO J 9:765–770

Horoszewicz JS, Leong SS, Kawinski E, Karr JP, Rosenthal H, Chu TM, Mirand EA, Murphy GP (1983) LNCaP model of human prostatic carcinoma. Cancer Res 43:1809–1818

Hynes RO (1992) Integrins: versitility, modulation and signaling in cell adhesion. Cell 69:11–25

Isberg RR, Leong JM (1990) Multiple β_1 chain integrins are receptors for invasin a protein that promotes bacterial penetration into mammalian cells. Cell 861–871

Kaighn ME, Narayan KS, Ohnuki Y, Lechner JF, Jones LW (1979) Establishment and characterization of a human prostate carcinoma cell line (PC-3). Invest Urol 17:16–23

Kramer RH, Vu M, Cheng Y-F, Ramos DM (1991) Integrin expression in malignant melanoma. Cancer Metastasis Rev 10:49–59

La Flamme SE, Akiyama SK, Yamada KM (1992) Regulation of fibronectin receptor distribution. J Cell Biol 117:437–447

Lee EC, Lotz MM, Steele GD Jr, Mercurio AM (1992) The integrin $\alpha_6\beta_4$ is a laminin receptor. J Cell Biol 117:671–678

Liotta LA, Kohn E (1990) Cancer invasion and metastases. JAMA 263:1123–1126

Long MM, King VJ, Prasad KU, Freeman BA, Urry DW (1989) Elastin repeat peptide as chemoattractants for bovine aortic endothelial cells. J Cell Physiol 140:512–518

Passaniti A, Issacs JT, Haney JA, Adler SW, Cujdik TJ, Long PV, Kleinman HK (1992) Stimulation of human prostatic carcinoma tumor growth in athymic mice and control of migration in culture be extracellular matrix. Int J Cancer 51:318–324

Plantefaber LC, Hynes RO (1989) Changes in integrin receptors on oncogenically transformed cells. Cell 56:281–290

Pretlow TG, Delmoro CM, Dilley GG, Spadafora CG, Pretlow TP (1991) Transplantation of human prostatic carcinoma into nude mice in matrigel. Cancer Res 51:3814–3817

Ruoslahti E, Giancotti FG (1989) Integrins and tumor cell dissemination. Cancer Cells 1:119–126

Shevrin DH, Kikreja SC, Vincic B, Lad TE (1986) Patterns of spontaneous metastasis by human prostate cancer grown in athymic nude mice. Proc Am Assoc Cancer Res 27:256A

Shevrin DH, Kukreja SC, Ghosh L, Lad TE (1988) Development of skeletal metastasis by human prostate cancer in athymic nude mice. Clin Exp Metastasis 6:401–409

Smith JW, Vestal DJ, Irwin SV, Burke TA, Cheresh DA (1990) Purification and functional characterization of integrin $\alpha_v\beta_5$. J Biol Chem 265:11008–11013

Sonnenberg A, Janssen H, Hogervorst V, Calafat J, Hilgers J (1987) A complex of platelet glycoproteins Ic and IIa identified by a rat monoclonal antibody. J Biol Chem 262:10376–10383

Sonnenberg A, Moddermann PW, Hogervorst F (1988) Laminin receptor on platelets is the integrin VLA-6. Nature 336:487–489

Stone KR, Mickey DD, Wunderli H, Mickey GH, Paulson DF (1978) Isolation of a human prostate carcinoma cell line (DU145). Int J Cancer 21:274–281

Vogel BE, Tarone G, Giancotti FG, Gailit J, Ruoslahti E (1990) A novel fibronectin receptor with an unexpected subunit composition ($\alpha_v\beta_1$). J Biol Chem 265:5934–5937

Wayner EA, Carter WG, Piotrowicz RS, Kunicki TJ (1988) The function of multiple extracellular matrix receptors in mediating cell adhesion to extracellular matrix: preparation of monoclonal antibodies to the fibronectin receptor that specifically inhibit cell adhesion to fibronectin and react with platelet glycoproteins Ic-IIa. J Cell Biol 107:1881–1891

Wayner EA, Garcia-Pardo A, Humphries MJ, McDonald JA, Carter WG (1989) Identification and characterization of the T lymphocyte adhesion receptor for an alternative cell attachment domain (CS-1) in plasma fibronectin. J Cell Biol 1321–1330

Wayner EA, Orlando RA, Cheresh DA (1991) Integrins $\alpha_v\beta_3$ and $\alpha_v\beta_5$ contribute to cell attachment to vitronectin but differentially distribute on the cell surface. J Cell Biol 113:919–929

Young IT (1977) Proof without prejudice: use of the Kolmogorov-Smirnov test for the analysis of histograms from flow systems and other sources. J Histochem Cytochem 25:935–941