[CANCER RESEARCH 61, 3308–3313, April 15, 2001]

Synthetic Peptides Inhibit Adhesion of Human Tumor Cells to Extracellular Matrix Proteins[1]

Ian B. DeRoock, Michael E. Pennington, Thomas C. Sroka, Kit S. Lam,[2] G. Tim Bowden, Elisabeth L. Bair, and Anne E. Cress[3]

Department of Radiation Oncology [I. B. D. , M. E. P. , T. C. S., K. S. L., G. T. B., E. L. B.] and Department of Molecular and Cellular Biology and Department of Medicine [A. E. C.], The Arizona Cancer Center, University of Arizona, Tucson, Arizona 85724-5024

ABSTRACT

Human tumor cell progression and metastasis are partially dependent on the ability of a tumor cell to adhere to the proteins of the extracellular matrix (ECM) and survive at the distant location. Six novel D-amino acid-containing peptides were analyzed for their ability to adhere to human prostate tumor cells, support tumor cell adhesion, and inhibit tumor cell adhesion to ECM proteins or human dermal fibroblasts. Of these, two peptides called RZ-3 (kmviywkag) and HYD-1 (kikmviswkg) bound to tumor cell surfaces and compared favorably with the previously reported AG-73 (RKRLQVQLSIRT) L-amino acid peptide, as determined by fluorescence-activated cell sorting analysis. A scrambled peptide derivative of HYD-1, called HYDS-1 (wiksmkivkg), was not active. The RZ-3, HYD-1, and AG-73 peptides supported maximal cancer cell adhesion at 5 μg, 10 μg, and 50 μg/well, respectively. The ECM proteins fibronectin, laminin 1, and collagen IV supported maximal cell adhesion at 1 μg, >10 μg, and 50 μg/well, respectively. Prostate tumor cell adhesion to immobilized RZ-3 and HYD-1 peptides was inhibited by α2–6- and β1-integrin-blocking antibodies. Conversely, tumor cell adhesion to a β1-integrin-specific antibody was blocked by both RZ-3 and HYD-1. Epithelial cell adhesion to dermal fibroblasts was inhibited by HYD-1 and unaffected by the scrambled peptide, HYDS-1. Cell adhesion to immobilized peptides was unaffected by EDTA. The soluble RZ-3 and HYD-1 peptides inhibited tumor cell adhesion to each of the immobilized four ECM proteins (1.0 μg/well) in a time- and concentration-dependent manner. The IC_{50} of the RZ-3 peptide for blocking adhesion to fibronectin, laminin 1, laminin 5, and collagen IV was 2.4 μg, 1.8 μg, 4.6 μg, and 2.8 μg/well, respectively. The IC_{50} of the HYD-1 peptide for blocking adhesion to fibronectin, laminin 1, laminin 5, and collagen IV was 6.9 μg, 5.7 μg, >10 μg, and 6.2 μg/well, respectively. Taken together, these results indicate that RZ-3 and HYD-1 are biologically active D-amino acid-containing peptides that can themselves support tumor cell adhesion and can inhibit tumor cell adhesion to immobilized ECM proteins or dermal fibroblasts.

INTRODUCTION

Tumor cell adhesion to the ECM[4] within tissues greatly influences the ability of a malignant cell to invade and metastasize to outlying tissues (reviewed in Ref. 1). Furthermore, the survival of the metastasized tumor cell depends in part upon the activity of the ECM receptors (2–7). Cell adhesion and the accompanying intermediate filaments in tumor cells are also an important factor in resistance to the killing effects of several chemotherapeutic drugs. Fibronectin-mediated adhesion of myeloma cells confers a survival advantage in a phenomenon known as CAM-DR (8–12). The pivotal role of adhesion modulation in both the tumor cell metastasis and their survival prompted us to characterize tumor cell adhesion peptides for their ability to alter cell adhesion to the ECM.

The proteins of the ECM consist of type I and IV collagens, laminins, heparin sulfate proteoglycan, fibronectin, and other noncollagenous glycoproteins (13). Cell adhesion to the laminins, fibronectin, and collagens is mediated in part by a group of heterodimeric transmembrane proteins called integrins, which are composed of a noncovalently associated α and β subunit that define the integrin-ligand specificity (14). The integrins $\alpha_6\beta_1$, $\alpha_3\beta_1$, and $\alpha_6\beta_4$ are laminin receptors (15, 16). These integrins in particular are associated with epithelial tumor progression in prostate, breast, colon, pancreatic carcinomas, head and neck tumors, and melanoma (17–21). Prostate micrometastases continue to express the α6 integrin, whereas other cell adhesion molecule expression is suppressed (22).

Previous attempts to block adhesion of cells to the ECM (*i.e.*, laminin 1, laminin 5, or collagen) have been accomplished using integrin-specific, function-blocking monoclonal antibodies or peptide mimics of the natural ECM protein (23–25); *e.g.*, human melanoma cells coinjected into nude mice with an anti-α6 antibody have a lowered ability to metastasize (26). Similarly, the $\alpha_1\beta_1$ and $\alpha_2\beta_1$ integrins are collagen receptors, and antibodies to these integrins reduced invasion of basement membranes (27). Biologically active adhesion peptides derived from specific regions of the ECM protein laminin 1 have had reported effects on a variety of biological processes including invasion, metastasis, migration, and matrix metalloproteinase expression (28–40).

Our previous work (41) identified several L-amino acid-containing peptide candidates as antiadhesive agents by selecting peptides based upon the ability of the prostate tumor cells to bind to the immobilized peptide. Further screening with a D-amino acid library yielded several new peptide candidates. Adhesion and biological properties of these peptides are analyzed in this study, and a hybrid peptide is proposed and further tested. The identification of D-amino acid peptides for the interruption of cell adhesion to ECM proteins or dermal fibroblasts should be useful in the increased killing of distant metastases, as well as providing insight into regulation of integrin-ligand interactions.

MATERIALS AND METHODS

Cell Line and Culture Conditions. The DU^{145} human prostate carcinoma cell line (American Type Culture Collection, Rockville, MD) originated from a prostate carcinoma brain metastasis. The DU-H cells were selected previously (42) using FACS for high α6 integrin expression. DU-H cells were maintained in DMEM medium with 10% fetal bovine serum (Life Technologies, Inc.) at 37°C in a humidified atmosphere of 95% air, 5% CO_2. The human oral squamous cell carcinoma line, SCC-25, was obtained from American Type Culture Collection. HFF were obtained as described previously (43). SCC-25 and HFF cells were maintained in DMEM:Ham's F-12 purchased from Life Technologies, Inc. supplemented with 10% fetal bovine serum from Gemini-Bio-Products (Calabasas, CA), 0.1% hydrocortisone, and penicillin (100 units/ml)/streptomycin (100 units/ml) in a humidified incubator at 37°C and 5% CO_2. Tissue-culture plastic ware was purchased from Costar (Cambridge, MA).

Received 1/19/00; accepted 2/19/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by NIH Grants CA 56666, CA 75152, CA 23074, ADCRC 9908, 9829.

[2] Present address: University of California Davis Cancer Center, Department of Internal Medicine, University of California, Davis, 4501 X Street, Sacramento, CA 95817.

[3] To whom requests for reprints should be addressed, at Arizona Cancer Center, University of Arizona, 1515 N. Campbell Avenue, Tucson, AZ 85724. Phone: (520) 626-7553; Fax: (520) 626-4979; E-mail: cress@azcc.arizona.edu.

[4] The abbreviations used are: ECM, extracellular matrix; CAM-DR, cell adhesion-mediated drug resistance; FACS, fluorescence-activated cell sorting; MPF, mean peak of fluorescence; HFF, human foreskin fibroblasts.

Synthetic Peptides, Fibronectin, Laminin 1, Laminin 5, and Collagen IV. The RZ series of peptides was found using the "one-bead one-compound" combinatorial library method and a D-isomer octyl-mer peptide library, as described previously by us (41). The amino acid sequences of the peptides are: RZ-3, kmviywkag; RZ-4, kggrhykfg; RZ-6, arkfkglig; and RZ-12, yiknrkhhg. The HYD-1 peptide (kikmviswkg) was generated by overlapping the positive adhesion peptides and postulating a hybrid sequence. The HYDS-1 scrambled peptide (wiksmkivkg) was generated by random ordering of the HYD-1 peptide sequence. The six peptides were synthesized with a biotin at the NH_2 terminus and purified by Molecular Resources Core Facility, Colorado State University, Department of Biochemistry, Ft. Collins, Colorado. The human fibronectin and mouse collagen IV was obtained from Life Technologies, Inc. The laminin 1 was obtained from Becton Dickinson Corp., Franklin Lakes, NJ and is derived from the Engelbreth-Holm-Swarm mouse tumor. The laminin 5 was obtained from the matrix of HaCaT cells (Dr. Norbert Fusenig, German Cancer Research Center, University of Heidelberg, Heidelberg, Germany; (Ref. 44).

Antibodies. All of the antibodies used were integrin function-blocking reagents known to react with human integrin. The mouse monoclonal antibodies used for the blocking studies were as follows: P4C10, anti-β1 integrin; P1E6, anti-α2 integrin; P1B5, anti-α3 integrin; and P1D6, anti-α5 integrin (all of these from Life Technologies, Inc.). NKI-GoH3, an anti-α6 integrin subunit rat antibody, was purchased from Serotec Inc. (Raleigh, NC). Mouse preimmune IgG was purchased from ICN Biomedicals Inc. (Aurora, OH). The β1 antibody used in the adhesion assay was TS2/16, obtained from the American Type Culture Collection.

FACS Analysis and Confocal Microscopy. The seven peptides were synthesized with a biotin at the NH_2 terminus. The peptides were then allowed to bind to neuralite avidin conjugated with Bodipy (Molecular Probes, Eugene, OR), and the tetrameric peptide-avidin complexes were incubated with the cells. The nonspecific binding was determined using the neuralite avidin-Bodipy alone incubated with the cells. Suspended DU-H cells (1×10^6) in 1 ml of IDMEM without serum were incubated with 10 μg of peptide and 10 μg of Bodipy conjugated to neuralite avidin at 4°C in the dark for 30 min. The cells were washed several times with IDMEM without serum and then analyzed for fluorescence on a FACStarPlus (Becton Dickinson) or examined using a laser scanning confocal microscope (LSM 10; Zeiss). Digital images were collected on each Z-series using identical contrast and brightness settings.

Cell Adhesion Assay and Peptide Inhibition of Adhesion. Ten μg of ligand or 20 μg of neuralite avidin (Molecular Probes, Inc.) was dissolved in 1 ml of distilled water, and 100 μl of solution was added to each well of a tissue culture 96-multiwell plate (Falcon, Franklin Lakes, NJ). The solution of either ECM protein or neuralite avidin was allowed to dry overnight. Use of neuralite avidin ensures maximum binding of the biotinylated peptide to the surface, avoiding the variability of peptide coating. In the β1 antibody adhesion assay, the TS2/16 antibody (2 μg/ml) was coated for 18 h in the HEPES buffer at 4C. The wells for all of the substrates were then blocked with 100 μl of 1% BSA for 1 h. The wells were washed with HEPES buffer, and varying amounts of peptide were added in each well. After 1 h of peptide incubation, wells were washed with IDMEM without serum, and suspended DU-H cells (5×10^4) in serum-free IDMEM were added in each well. The cells were allowed to adhere for 60 min at 37°C. The wells were washed three times with HEPES buffer and fixed with 2.5% formaldehyde in PBS. The cells were then stained with 0.5% crystal violet in 20% (v/v) methanol/water and viewed under a microscope. The amount of bound cells was estimated by solubilizing the dye using 0.1 M sodium citrate and reading the absorbance at 570 nm. Triplicate determinations were done at each data point.

Epithelial and Stromal Cell Adhesion Assay. HFF cells were used at 3×10^5 cells/well in 6-well plates in serum-free medium and allowed to attach at 37°C. After 24 h, the medium was removed, the well was washed once with PBS, and 3×10^5 SCC-25 cells were placed on top of the HFF monolayer. The SCC-25 cells were added using 1 ml of serum-free medium containing either 40 μg/ml HYD-1, 40 μg/ml HYDS-1, or no treatment. The number of SCC-25 unable to attach were recovered by a PBS wash and counted every h for 3 h using a hemocytometer. The determinations were done in triplicate.

RESULTS

RZ-3, HYD-1, and AG-73 Bind to Prostate Tumor Cell Surfaces. The RZ-3, RZ-4, RZ-6, RZ-12, and HYD-1 peptides and the previously reported AG-73 peptide (32) were tested for their ability to bind to DU-H cells by FACS analysis. In Fig. 1 (*top panel*), the MPF for each peptide is shown and indicates that Bodipy neuralite avidin alone resulted in a barely detectable amount of nonspecific binding. The RZ-4, RZ-6, and RZ-12 peptides demonstrated slight activity above the background of Bodipy neuralite avidin alone. The RZ-3, HYD-1, and AG-73 peptides exhibited significantly higher amounts of cell binding as compared with Bodipy neuralite avidin alone. Use of the RZ-3 peptide resulted in a MPF of 350; HYD-1 resulted in a MPF of 1200. The previously reported AG-73 peptide (32) resulted in

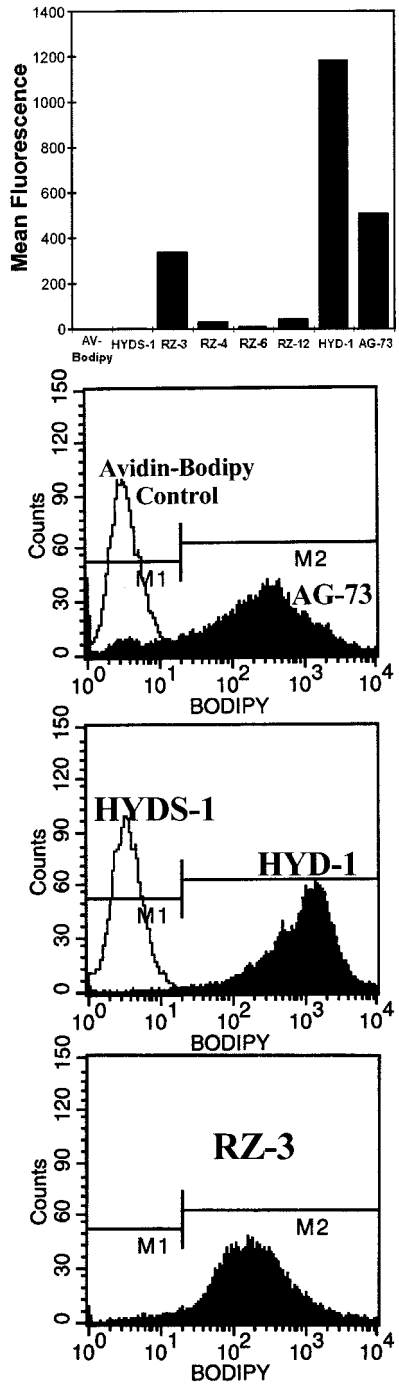

Fig. 1. FACS analysis of biotinylated peptides bound to DU-H prostate carcinoma cells. The cells were incubated with Bodipy-peptide complexes for 30 min, and the amount of peptide bound was estimated using FACS analysis. The MPF for each peptide from the resulting histograms are shown in the *top panel*. The representative histograms for the AG-73, HYD-1, HYDS-1, and the RZ-3 peptides are shown in the remaining *panels*.

a MPF of 500. The scrambled peptide, HYDS-1, had a MPF similar to the nonspecific binding detected by the Bodipy neuralite avidin alone, indicating that the scrambled peptide did not bind to the cells. The fluorescence histogram for each binding peptide is shown in the remaining panels of Fig. 1. All of the three peptides result in a normal distribution pattern of cell binding, indicating that there are cells within the populations that are low and high binders of the peptides. The AG-73 peptide had the broadest distribution pattern, followed by RZ-3 and HYD-1, respectively. Of the three peptides, the HYD-1 peptide displays the most uniform distribution. The distribution of both RZ-3 and HYD-1 peptides was also examined in live DU-H prostate tumor cells by confocal microscopy. The data in Fig. 2 indicate that the peptides remained on the surface of the cells during the 30-min incubation period. The confocal image also indicates that all of the cells were decorated with the peptide.

DU-H Prostate Tumor Cells Adhere to Immobilized Peptide and ECM Proteins. Using the three peptides that displayed the most cell-binding activity, RZ-3, HYD-1, and AG-73, the biological activities of the peptides were tested further. The ability of DU-H cells to adhere to the peptides immobilized on a plastic surface was compared with the ability of the cells to adhere to several immobilized natural ECM ligands, such as fibronectin, laminin 1, and collagen I (Fig. 3).

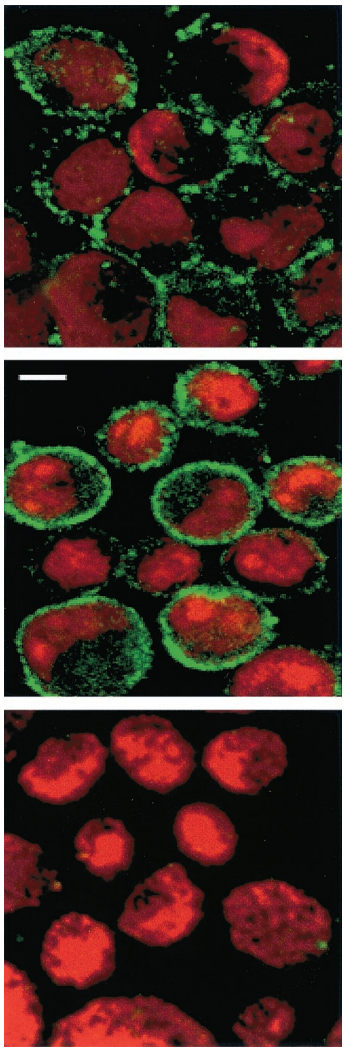

Fig. 2. Confocal microscopy of RZ-3 peptide binding to the surface of human prostate tumor cells. The DU-H cells were incubated with Bodipy-RZ-3 (*top panel*), Bodipy-HYD-1 (*middle panel*) peptide, or Bodipy alone (*bottom panel*) for 30 min, and the peptide (*green*) was observed using confocal microscopy on the live cells. The nuclei are stained with propidium iodide (*red*); *Bar*, 25 μm.

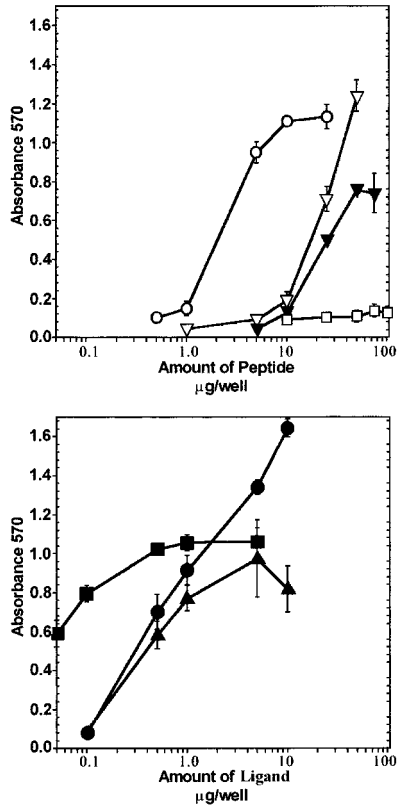

Fig. 3. Comparison of human prostate tumor cell adhesion to immobilized peptides and ECM proteins. The DU-H cells were allowed to attach to HYD-1 (○), AG-73 (▽), RZ-3 (▼), or HYDS-1 (□) peptide-coated 96-well microtiter plates (*top panel*) or fibronectin (■), laminin 1 (●), or collagen IV (▲) protein-coated 96-well microtiter plates (*bottom panel*). After a 60-min incubation at 37°C, the number of attached cells was determined by absorbance at 570 nm. Data are expressed as a mean of triplicate results; *error bars*, SD.

All of the three peptides supported cell adhesion within the 60-min incubation period (Fig. 3). The HYD-1 peptide promoted the most adhesion, with concentrations as low as 2 μg/well supporting cell attachment. Maximal cell adhesion to HYD-1 occurred at 10 μg/well. The scrambled peptide HYDS-1 did not support cell adhesion at any concentration tested (*i.e.*, 10–100 μg/well). The RZ-3 peptide required 10–50 μg/well for tumor cell adhesion. A comparison of the peptides indicates that 50% cell binding occurred using 1.5 μg, 20 μg, or 30 μg/well for HYD-1, AG-73, and RZ-3, respectively. Mixtures of the peptides were not synergistic for promoting adhesion (data not shown). All of the three ECM proteins are sufficient ligands for DU-H adhesion and can support adhesion in a similar manner at concentrations as low as 0.1 μg/well. A comparison of the ECM proteins indicates that 50% cell binding occurred using 0.1 μg, 0.6 μg, and 1.5 μg of fibronectin, laminin 1, and collagen IV, respectively. Taken together, a comparison of the ability of the peptides and the ECM proteins to support adhesion indicates that the HYD-1 peptide compares favorably with the natural ligands.

Tumor Cell Adhesion to Immobilized Peptides Is Integrin-dependent. Next, we tested if cell adhesion to the peptides or the natural ligands was dependent upon cations or whether the interaction could be blocked by integrin function-blocking antibodies.

Cell attachment to laminin 1 and fibronectin was inhibited by 3 mM EDTA as expected (Fig. 4), indicating that these interactions are conducted through an interaction that requires cations (45). In contrast, cell attachment to RZ-3 and HYD-1 was not affected by 3 mM EDTA, suggesting that the adhesive properties occur through an integrin interaction that does not require cations. This is consistent

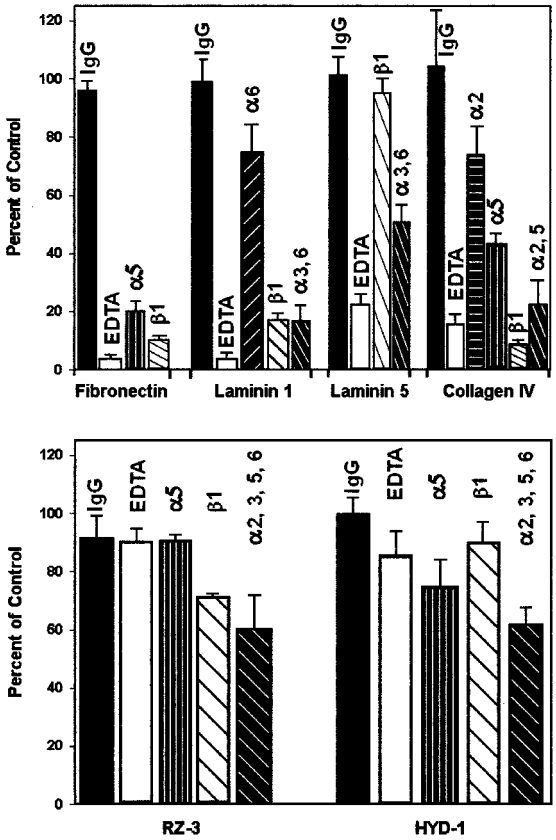

Fig. 4. Effect of antibodies and reagents on DU-H cell adhesion to peptides and ECM proteins. The DU-H cells were allowed to attach to fibronectin- (1 μg/well), laminin 1- (1 μg/well), collagen IV- (1 μg/well), HYD-1- (10 μg/well), or RZ-3- (50 μg/well) coated plates in the presence of 10 μg/ml IgG, 3 mM EDTA; anti-α2; anti-α5; anti-α3 and 6; anti-α2 and 5 integrin antibodies; or anti-β1 integrin antibody, as indicated. After 60 min of incubation at 37°C, the number of attached cells was determined by absorbance at 570 nm. Data are expressed as a mean of triplicate results; *error bars*, SD. The percentage of control is the absorbance value of cells with the function-blocking antibody divided by the absorbance value of cells without the function-blocking antibody $\times$ 100.

with a peptide interaction occurring independent of the calcium-binding motif. The calcium-binding motifs of the α integrin subunit lie on a surface of the integrin far from the ligand contact sites (45). Cell attachment to laminin 1 and fibronectin was not affected by the addition of 10 μg/ml heparin, suggesting that the cell adhesion to the immobilized matrix proteins was not because of the positive charge interactions with the cell surface. Similarly, attachment to RZ-3 and HYD-1 was unaffected by adding heparin (data not shown). Cell attachment to fibronectin was inhibited by the anti-α5 and the anti-β1 antibody, consistent with its known function as a ligand for the $\alpha_5\beta_1$ integrin. Cell attachment to laminin 1 was inhibited by anti-α3,6 and anti-β1 integrin antibodies, consistent with the fact that adhesion to laminin 1 is mediated by both the α3,6 and β1 integrins. Cell attachment to laminin 5 was partially inhibited by the α3,6 antibodies and unaffected by the β1-blocking antibody. A combination of the α2, 3, 5, and 6 integrin-blocking antibodies partially inhibited adhesion to immobilized HYD-1 or RZ-3 peptides. Although cell adhesion to HYD-1 was not inhibited by β1-blocking antibodies, HYD-1 peptide can inhibit cell adhesion to the β1 integrin-specific activating antibody TS2/16 (Fig. 5). Taken together, the data suggest that cell adhesion to the immobilized RZ-3 and HYD-1 are integrin dependent.

Inhibition of DU-H Cell Attachment to ECM Proteins. The next test of the biological properties of the peptides was to determine whether the soluble peptides could inhibit DU-H adhesion to the immobilized ECM proteins.

The DU-H cell attachment to immobilized laminin 1 and fibronectin was decreased in a concentration-dependent manner when HYD-1 or RZ-3 was added to the wells (Fig. 6). The RZ-3 peptide was the most effective peptide for inhibiting attachment to all of the ECM proteins, including laminin 5, as judged by comparing the concentration of peptide required to inhibit 50% of the cell adhesion (IC_{50}) to the respective ECM protein (Table 1). The previously reported AG-73 peptide (32) inhibited cell adhesion to fibronectin, requiring approximately 9 μg/well, and did not inhibit cell adhesion to laminin 1 or collagen IV. The HYD-1 peptide inhibited cell adhesion to laminin 1, fibronectin, and collagen IV in a similar manner. The HYD-1 peptide inhibition of cell adhesion showed a threshold response in that greater than 2 μg/well of peptide was required before an inhibition of cell adhesion was detected. The scrambled HYDS-1 peptide was not able to inhibit adhesion to any ECM protein. A comparison of the IC_{50} values of the peptides reveals that the most potent inhibitor for attachment to all of the three ECM proteins is RZ-3.

Inhibition of SCC-25 and HFF Coculture Interaction by HYD-1 Peptide. The biological relevance of the adhesion-blocking ability of the HYD-1 peptide was tested using an epithelial-fibroblast coculture model system. Epithelial stromal interactions are increasingly recognized as determinants of tumor progression. The adhesion of SCC-25 cells to a HFF monolayer is a time-dependent process that was maximal within 1 h of incubation at 37°C (Fig. 7). The HYD-1 peptide inhibited approximately half of the SCC-25 cells from attaching to the HFF monolayer. The inhibition of SCC-25 adhesion was maintained over the 3-h period of the assay. The scrambled form of the peptide, HYDS-1, was ineffective in altering adhesion.

DISCUSSION

Using a previously described method (41) to isolate candidate peptides for biological activity with the α6 integrin, we report the existence of two bioactive D-amino acid-containing peptides called RZ-3 and HYD-1. These peptides attach to the surfaces of live tumor cells, can themselves support cell attachment, can inhibit attachment to ECM proteins such as fibronectin, laminin 1, laminin 5, and collagen IV, and can inhibit epithelial stromal adhesion interactions.

The inhibition of tumor cell adhesion to a number of ECM proteins was observed with both peptides. In addition, the integrin-blocking antibodies only partially inhibited the adhesion of the cells to the peptides. Taken together, these data suggest that the peptides can affect adhesion processes that are both integrin dependent and independent. This fact may have significance for both understanding

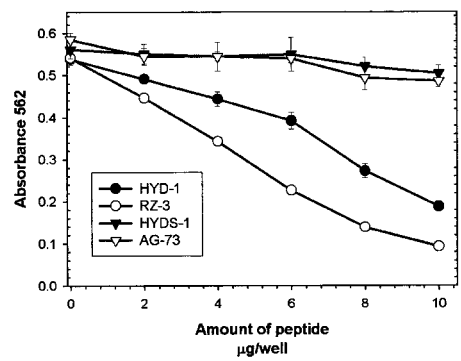

Fig. 5. Inhibition of DU145 cell adhesion to immobilized β1 integrin antibody by peptides. The cells were allowed to attach to the immobilized β1 integrin antibody called TS2/16 (2 μg/well) in the presence of increasing concentrations of soluble peptide (HYD-1, RZ-3, HYDS-1, or AG-73) using serum-free medium. After 1 h at 37°C, the number of attached cells was determined by absorbance at 570 nm. Data are expressed as a mean of triplicate results; *error bars*, SD.

cross-talking pathways of cell adhesion to the ECM and interrupting adhesion-dependent biological events. The broad nature of the inhibition could prove useful in disabling the varied adhesion events that are activated within human tumor cell populations. It should be noted that these peptides do not show homology to any known proteins in the SwissProt database using a Basic Local Alignment Search Tool for Protein (data not shown).

The potential use of the peptides could include the prevention of tumor cell adhesion *in vivo*. Preliminary experiments using a mouse human tumor xenograft model system (46) suggest that the peptides themselves are not toxic to animals (data not shown). The alteration of tumor cell adhesion could be of benefit for preventing metastasis as well as increasing the killing of tumor cells that are refractory to treatment. The peptides may be particularly useful in human tumors with a wild-type p53 function because clustering of integrin in tumor cells containing wild-type p53 can trigger apoptosis (7). Our preliminary experiments indicate that the altering of attachment can sensitize the tumor cells to the killing effects of ionizing radiation (data not shown). We are currently testing both the efficacy of the peptides to inhibit tumor cell adhesion *in vivo* and the potential for synergistic lethality with DNA-damaging agents *in vivo*.

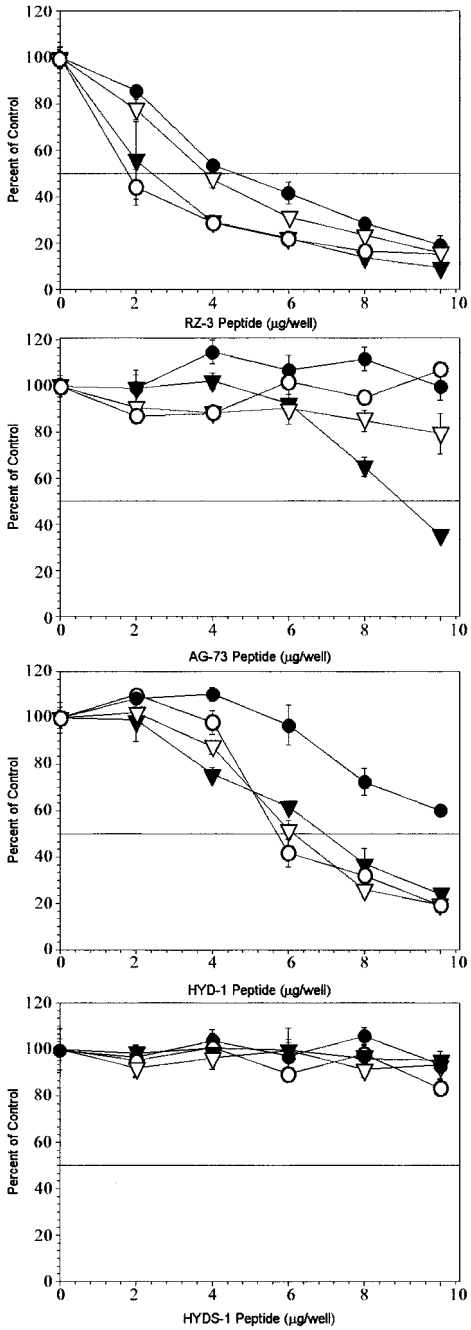

Fig. 6. Inhibition of DU-H cell adhesion to ECM proteins by peptides. The cells were allowed to attach to laminin 1- (▼, 1 μg/well), fibronectin- (○, 1 μg/well), laminin 5- (●, 1 μg/well), or collagen IV- (▽, 1 μg/well) coated 96-well microtiter plates in the presence of various amounts the RZ-3, HYD-1, or HYDS-1 peptides using serum-free media. After a 60-min incubation at 37°C, the number of attached cells was determined by absorbance at 570 nm. Data are expressed as a mean of triplicate results. The percentage of control is the absorbance value of cells with the peptide divided by the absorbance value of the cells without the peptide × 100.

Table 1 *Concentration of peptide for IC_{50} of cell adhesion to ECM proteins (μg/well)*

	Fibronectin	Laminin 1	Laminin 5	Collagen IV
RZ-3	2.4	1.8	4.6	2.8
HYD-1	6.9	5.7	>10	6.2
HYDS-1	No inhibition	No inhibition	No inhibition	No inhibition
AG-73	9.0	No inhibition	No inhibition	>10

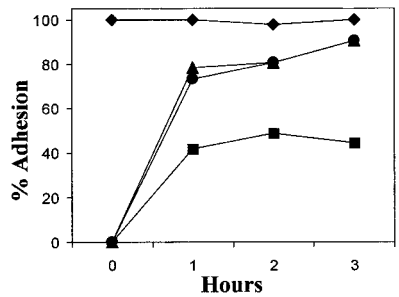

Fig. 7. Inhibition of SCC-25 cell adhesion to HFF monolayers by peptides. Approximately 3×10^5 SCC-25 cells were plated onto HFF monolayers in the absence (●) or presence of the HYD-1 (■) and HYDS-1 (▲) peptides. After the indicated times of incubation, the number of SCC-25 cells unable to attach was determined using a hemocytometer. The number of HFF cells unable to attach was also determined in the presence of the HYD-1 peptide (◆). A minimum of three determinations were done at each time point. The percentage of adhesion is the number of SCC-25 cells adhering to the HFF monolayer divided by the number of SCC-25 cells added to the wells × 100.

It has been demonstrated that fibronectin-mediated adhesion confers a survival advantage for myeloma cells acutely exposed to cytotoxic drugs in a phenomenon known as CAM-DR (11, 12). Myeloma cells within the bone marrow are adherent to fibronectin and are thought to serve as a reservoir of tumor cells that are difficult to eradicate. The antiadhesive peptides HYD-1 and RZ-3 may be lead candidates to overcome CAM-DR.

The role of the ECM as a cell survival factor has been demonstrated in both normal cell types and fibroblasts. The ECM is critical for normal survival signals, and the loss of the ECM will result in normal cell death in a process termed anoikis (47–50). However, during transformation, normal cells down-regulate many of their adhesive interactions and display anchorage-independent cell growth. Although the majority of the adhesion events are suppressed during transformation, selected adhesion events are preserved; *e.g.*, in prostate cancer progression, the majority of the integrin ECM receptors and the E-cadherin class of adhesion molecules are suppressed (51), whereas the laminin receptors and *N*-cadherin remain expressed (18, 52). The preserved adhesion events may be important targets for inactivating the known survival advantage of invasive or metastatic tumor cells.

REFERENCES

1. Boudreau, N., and Bissell, M. J. Extracellular matrix signaling: integration of form and function in normal and malignant cells. Curr. Opin. Cell Biol., *10:* 640–646, 1998.

2. Wewer, U. M., Shaw, L. M., Albrechtsen, R., and Mercurio, A. M. The integrin $\alpha 6\beta 1$ promotes the survival of metastatic human breast carcinoma cells in mice. Am. J. Pathol., *151:* 1191–1198, 1997.
3. Meerschaert, J., Vrtis, R. F., Shikama, Y., Sedgwick, J. B., Busse, W. W., and Mosher, D. F. Engagement of $\alpha 4\beta 7$ integrins by monoclonal antibodies or ligands enhances survival of human eosinophils *in vitro*. J. Immunol., *163:* 6217–6227, 1999.
4. Sethi, T., Rintoul, R. C., Moore, S. M., MacKinnon, A. C., Salter, D., Choo, C., Chilvers, E. R., Dransfield, I., Donnelly, S. C., and Strieter, R. Extracellular matrix proteins protect small cell lung cancer cells against apoptosis: a mechanism for small cell lung cancer growth and drug resistance *in vivo*. Nat. Med., *5:* 662–668, 1999.
5. Streuli, C. H., and Gilmore, A. P. Adhesion-mediated signaling in the regulation of mammary epithelial cell survival. J. Mammary Gland Biol. Neoplasia, *4:* 183–191, 1999.
6. Brizzi, M. F., Defilippi, P., Rosso, A., Venturino, M., Garbarino, G., Miyajima, A., Silengo, L., Tarone, G., and Pegoraro, L. Integrin-mediated adhesion of endothelial cells induces JAK2 and STAT5A activation: role in the control of c-fos gene expression. Mol. Biol. Cell, *10:* 3463–3471, 1999.
7. Bachelder, R. E., Ribick, M. J., Marchetti, A., Falcioni, R., Soddu, S., Davis, K. R., and Mercurio, A. M. p53 inhibits $\alpha 6\beta 4$ integrin survival signaling by promoting the caspase 3-dependent cleavage of Akt/Pkb. J. Cell Biol., *147:* 1063–1072, 1999.
8. Cress, A. E., and Dalton, W. S. Multiple drug resistance and intermediate filaments. Cancer Metastasis Rev., *15:* 499–506, 1996.
9. St. Croix, B., Man, S., and Kerbel, R. S. Reversal of intrinsic and acquired forms of drug resistance by hyaluronidase treatment of solid tumors. Cancer Lett., *131:* 35–44, 1998.
10. Kerbel, R. S., Rak, J., Kobayashi, H., Man, M. S., St Croix, B., and Graham, C. H. Multicellular resistance: a new paradigm to explain aspects of acquired drug resistance of solid tumors. Cold Spring Harbor Symp. Quant. Biol., *59:* 661–672, 1994.
11. Damiano, J. S., Cress, A. E., Hazlehurst, L. A., Shtil, A. A., and Dalton, W. S. Cell adhesion mediated drug resistance (CAM-DR): role of integrins and resistance to apoptosis in human myeloma cell lines. Blood, *93:* 1658–1667, 1999.
12. Hazlehurst, L. A., Foley, N. E., Gleason-Guzman, M. C., Hacker, M. P., Cress, A. E., Greenberger, L. W., De Jong, M. C., and Dalton, W. S. Multiple mechanisms confer drug resistance to mitoxantrone in the human 8226 myeloma cell line. Cancer Res., *59:* 1021–1028, 1999.
13. Hay, E. D. Cell Biology of the Extracellular Matrix, Vol. 2. New York: Plenum Press, 1991.
14. Hynes, R. O. Integrins: versatility, modulation, and signaling in cell adhesion. Cell, *69:* 11–25, 1992.
15. Lee, E. C., Lotz, M. M., Steele, G. D., Jr., and Mercurio, A. M. The integrin $\alpha 6\beta 4$ is a laminin receptor. J. Cell Biol., *117:* 671–678, 1992.
16. Mercurio, A. M., and Shaw, L. M. Laminin binding proteins. BioEssays, *13:* 469–473, 1991.
17. Cress, A. E., Rabinovitz, I., Zhu, W., and Nagle, R. B. The $\alpha 6\beta 1$ and $\alpha 6\beta 4$ integrins in human prostate cancer progression. Cancer Metastasis Rev., *14:* 219–228, 1995.
18. Friedrichs, K., Ruiz, P., Franke, F., Gille, I., Terpe, H. J., and Imhof, B. A. High expression level of $\alpha 6$ integrin in human breast carcinoma is correlated with reduced survival. Cancer Res., *55:* 901–906, 1995.
19. Rabinovitz, I., and Mercurio, A. M. The integrin $\alpha 6\beta 4$ and the biology of carcinoma [Review]. Biochem. Cell Biol., *74:* 811–821, 1996.
20. Rosendahl, A., Neumann, K., Chaloupka, B., Rothmund, M., and Weinel, R. J. Expression and distribution of VLA receptors in the pancreas: an immunohistochemical study. Pancreas, *8:* 711–718, 1993.
21. Carey, T. E., Laurikainen, L., Ptok, A., Reinke, T., Linder, K., Nair, T. S., and Marcelo, C. Culture conditions affect expression of the $\alpha 6\beta 4$ integrin associated with aggressive behavior in head and neck cancer. Adv. Exp. Med. Biol., *320:* 69–79, 1992.
22. Putz, E., Witter, K., Offner, S., Stosiek, P., Zippelius, A., Johnson, J., Zahn, R., Riethmuller, G., and Pantel, K. Phenotypic characteristics of cell lines derived from disseminated cancer cells in bone marrow of patients with solid epithelial tumors: establishment of working models for human micrometastases. Cancer Res., *59:* 241–248, 1999.
23. Weinel, R. J., Rosendahl, A., Neumann, K., Chaloupka, B., Erb, D., Rothmund, M., and Santoso, S. Expression and function of VLA-$\alpha 2$, -$\alpha 3$, -$\alpha 5$ and -$\alpha 6$-integrin receptors in pancreatic carcinoma. Int. J. Cancer, *52:* 827–833, 1992.
24. Niessen, C. M., Hogervorst, F., Jaspars, L. H., de Melker, A. A., Delwel, G. O., Hulsman, E. H., Kuikman, I., and Sonnenberg, A. The $\alpha 6\beta 4$ integrin is a receptor for both laminin and kalinin. Exp. Cell Res., *211:* 360–367, 1994.
25. Brown, J. C., and Goodman, S. L. Different cellular receptors for human placental laminin and murine EHS laminin. FEBS Lett., *282:* 5–8, 1991.
26. Ruiz, P., Dunon, D., Sonnenberg, A., and Imhof, B. A. Suppression of mouse melanoma metastasis by EA-1, a monoclonal antibody specific for $\alpha 6$ integrins. Cell Adhes. Commun., *1:* 67–81, 1993.
27. Lochter, A., Navre, M., Werb, Z., and Bissell, M. J. $\alpha 1$ and $\alpha 2$ integrins mediate invasive activity of mouse mammary carcinoma cells through regulation of stromelysin-1 expression. Mol. Biol. Cell, *10:* 271–282, 1999.
28. Kibbey, M. C., Grant, D. S., and Kleinman, H. K. Role of the SIKVAV site of laminin in promotion of angiogenesis and tumor growth: an *in vivo* Matrigel model. J. Natl. Cancer Inst. (Bethesda), *84:* 1633–1638, 1992.
29. Grant, D. S., Kinsella, J. L., Fridman, R., Auerbach, R., Piasecki, B. A., Yamada, Y., Zain, M., and Kleinman, H. K. Interaction of endothelial cells with a laminin A chain peptide (SIKVAV) *in vitro* and induction of angiogenic behavior *in vivo*. J. Cell. Physiol., *153:* 614–625, 1992.
30. Bresalier, R. S., Schwartz, B., Kim, Y. S., Duh, Q. Y., Kleinman, H. K., and Sullam, P. M. The laminin $\alpha 1$ chain Ile-Lys-Val-Ala-Val (IKVAV)-containing peptide promotes liver colonization by human colon cancer cells. Cancer Res., *55:* 2476–2480, 1995.
31. Nomizu, M., Kuratomi, Y., Song, S. Y., Ponce, M. L., Hoffman, M. P., Powell, S. K., Miyoshi, K., Otaka, A., Kleinman, H. K., and Yamada, Y. Identification of cell binding sequences in mouse laminin $\gamma 1$ chain by systematic peptide screening. J. Biol. Chem., *272:* 32198–32205, 1997.
32. Nomizu, M., Kim, W. H., Yamamura, K., Utani, A., Song, S. Y., Otaka, A., Roller, P. P., Kleinman, H. K., and Yamada, Y. Identification of cell binding sites in the laminin $\alpha 1$ chain carboxyl-terminal globular domain by systematic screening of synthetic peptides. J. Biol. Chem., *270:* 20583–20590, 1995.
33. Skubitz, A. P., Letourneau, P. C., Wayner, E., and Furcht, L. T. Synthetic peptides from the carboxy-terminal globular domain of the A chain of laminin: their ability to promote cell adhesion and neurite outgrowth, and interact with heparin and the $\beta 1$ integrin subunit. J. Cell Biol., *115:* 1137–1148, 1991.
34. Song, S. Y., Nomizu, M., Yamada, Y., and Kleinman, H. K. Liver metastasis formation by laminin-1 peptide (LQVQLSIR)-adhesion selected B16–F10 melanoma cells. Int. J. Cancer, *71:* 436–441, 1997.
35. Graf, J., Ogle, R. C., Robey, F. A., Sasaki, M., Martin, G. R., Yamada, Y., and Kleinman, H. K. A pentapeptide from the laminin B1 chain mediates cell adhesion and binds the 67,000 laminin receptor. Biochemistry, *26:* 6896–6900, 1987.
36. Iwamoto, Y., Robey, F. A., Graf, J., Sasaki, M., Kleinman, H. K., Yamada, Y., and Martin, G. R. YIGSR, a synthetic laminin pentapeptide, inhibits experimental metastasis formation. Science (Wash DC), *238:* 1132–1134, 1987.
37. Sakamoto, N., Iwahana, M., Tanaka, N. G., and Osada, Y. Inhibition of angiogenesis and tumor growth by a synthetic laminin peptide, CDPGYIGSR-NH2. Cancer Res., *51:* 903–906, 1991.
38. Skubitz, A. P., McCarthy, J. B., Zhao, Q., Yi, X. Y., and Furcht, L. T. Definition of a sequence, RYVVLPR, within laminin peptide F-9 that mediates metastatic fibrosarcoma cell adhesion and spreading. Cancer Res., *50:* 7612–7622, 1990.
39. Kleinman, H. K., Graf, J., Iwamoto, Y., Sasaki, M., Schasteen, C. S., Yamada, Y., Martin, G. R., and Robey, F. A. Identification of a second active site in laminin for promotion of cell adhesion and migration and inhibition of *in vivo* melanoma lung colonization. Arch. Biochem. Biophys., *272:* 39–45, 1989.
40. Charonis, A. S., Skubitz, A. P., Koliakos, G. G., Reger, L. A., Dege, J., Vogel, A. M., Wohlhueter, R., and Furcht, L. T. A novel synthetic peptide from the B1 chain of laminin with heparin- binding and cell adhesion-promoting activities. J. Cell Biol., *107:* 1253–1260, 1988.
41. Pennington, M. E., Lam, K. S., and Cress, A. E. The use of a combinatorial library method to isolate human tumor cell adhesion peptides. Mol. Divers., *2:* 19–28, 1996.
42. Rabinovitz, I., Nagle, R. B., and Cress, A. E. Integrin $\alpha 6$ expression in human prostate carcinoma cells is associated with a migratory and invasive phenotype *in vitro* and *in vivo*. Clin. Exp. Metastasis, *13:* 481–491, 1995.
43. Borchers, A. H., Sanders, L. A., Powell, M. B., and Bowden, G. T. Melanocyte mediated paracrine induction of extracellular matrix degrading proteases in squamous cell carcinoma cells. Exp. Cell Res., *231:* 61–65, 1997.
44. Breitkreutz, D., Schoop, V. M., Mirancea, N., Baur, M., Stark, H. J., and Fusenig, N. E. Epidermal differentiation and basement membrane formation by HaCaT cells in surface transplants. Eur. J. Cell Biol., *75:* 273–286, 1998.
45. Loftus, J. C., and Liddington, R. C. Cell adhesion in vascular biology. New insights into integrin-ligand interaction. J. Clin. Investig., *99:* 2302–2306, 1997.
46. McCandless, J. R., Cress, A. E., Rabinovitz, I., Payne, C. M., Bowden, G. T., Knox, J. D., and Nagle, R. B. A human xenograft model for testing early events of epithelial neoplastic invasion. Int. J. Oncol., *10:* 279–285, 1997.
47. Ilic, D., Almeida, E. A., Schlaepfer, D. D., Dazin, P., Aizawa, S., and Damsky, C. H. Extracellular matrix survival signals transduced by focal adhesion kinase suppress p53-mediated apoptosis. J. Cell Biol., *143:* 547–560, 1998.
48. Frisch, S. M., and Ruoslahti, E. Integrins and anoikis. Curr. Opin. Cell Biol., *9:* 701–706, 1997.
49. Schwartz, M. A. Integrins, oncogenes, and anchorage independence. J. Cell Biol., *139:* 575–578, 1997.
50. Ruoslahti, E., and Reed, J. C. Anchorage dependence, integrins, and apoptosis. Cell, *77:* 477–478, 1994.
51. MacGrogan, D., and Bookstein, R. Tumor suppressor genes in prostate cancer. Semin. Cancer Biol., *8:* 11–19, 1997.
52. Tran, N. L., Nagle, R. B., Cress, A. E., and Heimark, R. L. N-Cadherin expression in human prostate carcinoma cell lines. An epithelial-mesenchymal transformation mediating adhesion with stromal cells. Am. J. Pathol., *155:* 787–798, 1999.

Synthetic Peptides Inhibit Adhesion of Human Tumor Cells to Extracellular Matrix Proteins

Ian B. DeRoock, Michael E. Pennington, Thomas C. Sroka, et al.

Cancer Res 2001;61:3308-3313.