and reducing agents, EDTA-Hin(139–190) cleaves double-stranded DNA at the recognition sequence of the Hin recombinase site, revealing the location of the NH_2-terminus of Hin(139–190). The coupling of a DNA-binding peptide to a metal chelator creates a hybrid peptide capable of cleaving specific sites on DNA. Design of other multifunctional peptides capable of recognizing specific substrates and chemical modification of those substrates could lead to reagents for use in chemistry, molecular biology, and medicine.

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YIGSR, a Synthetic Laminin Pentapeptide, Inhibits Experimental Metastasis Formation

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The invasion of tumor cells through basement membranes is a critical step in the formation of metastases. The binding of the malignant cells to laminin in the basement membranes allows their attachment and activates their invasiveness. Recently a synthetic nonapeptide from the B1 chain sequence of laminin was identified as a major site for cell binding. A pentapeptide within the nonapeptide sequence was found to reduce the formation of lung colonies in mice injected with melanoma cells and also to inhibit the invasiveness of the cells in vitro.

LAMININ, A BASEMENT MEMBRANE–specific glycoprotein, has various biological activities including promoting the attachment, growth, and differentiation of epithelial cells (*1*). It also appears to be involved in tumor cell invasion and metastasis. Malignant cells have more laminin on their surface, bind more laminin, and attach more readily to laminin (*2, 3*). Laminin increases their invasive and metastatic activity (*3, 4*) and induces the secretion of collagenase IV (*5*). These activities appear to involve the binding of laminin to a high affinity receptor on the cell surface (M_r = 67,000) (*6*), since proteolytic fragments of laminin ($M_r \cong 450{,}000$) that bind to the receptor and block the formation of metastases (*4, 7*).

Laminin is composed of three chains, A(400 kD), B1(230 kD), and B2(220 kD) chain (*8*), which are arranged in a cross-shaped structure (Fig. 1). We have cloned and sequenced the B1 chain (*9*), prepared synthetic peptides and peptide-specific antibodies, and used these to identify a sequence (CDPGYIGSR) (*10*) in the B1 chain which is active in cell attachment, chemotaxis, and binding to the laminin receptor (*11*). In this report, we have tested the nonapeptide (called peptide 11) and its amide form (peptide 11–amide) as well as other peptides in (i) an in vitro invasion assay (*12*) and (ii) a murine model of lung tumor colonization after the intravenous injection of B16F10 melanoma cells (*13*). The in vitro invasion assay measures the ability of cells to attach, degrade, and migrate through a reconstituted basement membrane matrix. By means of this assay, invasiveness has been found to strongly correlate with metastatic activity (*12*). We find that peptide 11 and its terminal pentapeptide YIGSR inhibit tumor cell invasion. In other studies on cell adhesion and receptor binding, we found that peptide 11–amide was more active, probably because it neutralized the negative charge on the arginine (*14*). The amide form also appeared to be more active in the in vitro invasion assay (Fig. 2). Other peptides of 19 to 22 amino acids in length from different domains in the B1 chain of laminin (peptides 1-7 in Fig. 1) were inactive (Fig. 2; P2 is shown). Similar findings on the activity of the peptides were obtained in the in vivo assay for lung tumor colonization (Fig. 3). Both peptide 11 and peptide 11–amide reduced the numbers of lung tumors (by 74% and >90%, respectively) when administered with B16F10 cells by tail vein injection into mice (Fig. 3). The inhibition of colonization

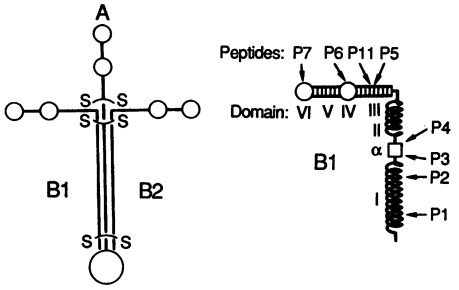

Fig. 1. Schematic model for the B1 chain of laminin. Seven structural domains in the B1 chain of laminin have been described and these are designated I–VI and α (*9*). The circles designate the globular regions of the laminin and the square designates an unusual cysteine-rich homologous repeat. P1 (residue 1593–1611), KQADEDIQGTQNLLTSIES; P2 (residue 1509–1529), KSGNASTPQQLQNLTEDIRER; P3 (residue 1395–1416), CRTDEGEKKCGGPGCGGLVTVA; P4 (residue 1363–1383), KLQSLDLSAAAQMTCGTPPGA; P5 (residue 960–978), NIDTTDPEACDKDTGRCLK; P6 (residue 615–634), KIPASSRCGNTVPDDDNQVV; P7 (residue 364–385), PERDIRDPNLCEPCTCDPAGSE; P11 (residue 925–933), CDPGYIGSR. Peptides were synthesized with an automated synthesizer, model 430 A (Applied Biosystems, Inc., Foster City, California). Their purity was checked by amino acid analyses and by high performance liquid chromatography.

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by the nonapeptide was dose-dependent (Fig. 4); lower doses (50 to 100 μg per mouse) inhibited the formation of the majority of the colonies in the lung but much higher doses (1 mg per mouse) were required for total blockade. To better define the active sequence, various peptides were tested at the high concentration (1 mg per mouse) for their ability to prevent colonization in the lung (Fig. 5). A peptide, NIDTTDPEACDKDTGRCLK, from another domain in laminin did not show any activity. CDPGYIGSR-NH_2 showed the greater inhibition of lung tumor colony formation (more than 90%). When the nonapeptide and cells were injected into separate tail veins, a similar degree of inhibition of lung tumors was observed, demonstrating that preincubation of the cells with the peptide is not required. The pentapeptide YIGSR and its amide form showed a comparable degree of inhibition. The amide form of the reverse sequence, RSGIY-NH_2, showed much lower activity. Pentapeptide YIGSE was less active, suggesting that arginine is important for activity. RGDS-NH_2 was also tested because GRGDS, a sequence from the cell-binding domain of fibronectin, has been reported to inhibit lung colonization by melanoma cells (*15*). This peptide reduced lung colonies by about 30% and RGDSGYIGSR-NH_2, which contains both the RGDS and the GYIGSR-NH_2 sequences, inhibited lung tumor colonization by about 50%. These data suggest that YIGSR from the B1 chain of laminin is more active than the fibronectin-derived sequences in inhibiting lung tumor colonization; the reason for this may be the higher affinity of laminin than fibronectin for receptor binding (*16*).

The inhibition of lung tumor colonization by the active peptides was not due to cytotoxicity since incubation of cells with peptide 11 or peptide 11-amide at 10 mg/ml for 20 minutes did not affect their subsequent proliferation rate or the final cell density attained on culture dishes (*17*). Furthermore, these peptides did not inhibit lung tumor colonization by altering the tumorigenicity of the cells. The weight of tumors which formed 14 days after melanoma cells were injected subcutaneously with or without 1 mg of these peptides were similar [CDPGYIGSR (mean ± SD = 1.2 ± 0.7 g, n = 6), its amide form (1.3 ± 0.7 g, n = 6), and control (1.0 ± 0.6 g, n = 6)]. Lastly, preincubation of the melanoma cells with the peptides did not show any effect on cell aggregation.

As there was an exact correlation between the inhibitory activities of the various peptides in the in vitro and in vivo assays, it seems likely that YIGSR inhibits lung tumor colony formation by blocking tumor cell invasion through basement membranes. In agreement with this conclusion is our finding that YIGSR is active in the attachment of fibrosarcoma HT-1080, Chinese hamster ovary (CHO) cells (*11*), and B16F10 cells (*18*). In addition, the pentapeptide elutes the 67-kD laminin cell surface receptor from a laminin affinity column (*11*). We have also directly tested this peptide on cellular activities implicated in invasion. YIGSR inhibits cell adhesion (60% at 100 μg/ml) and migration toward laminin (80% at 200 μg/ml), but has no effect on collagenase production at levels up to 300 μg/ml; 10 μg/ml of

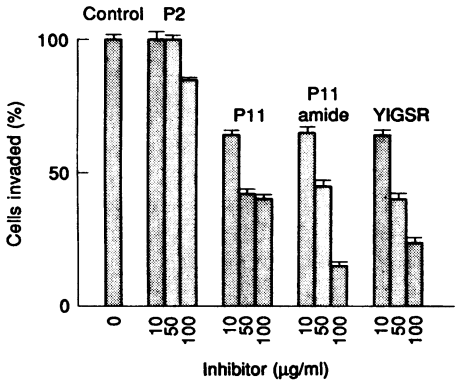

Fig. 2. Inhibition of B16F10 melanoma cell invasion in vitro by synthetic peptides of the B1 chain of laminin. B16F10 melanoma cells were obtained from I. J. Fidler, Houston, Texas, and were propagated under standard culture conditions. The chemoinvasion assay was carried out as described (*12*). Briefly, polycarbonate filters (8-μm pore size; Nuclepore, Pleasanton, California) were coated with 50 μg of a reconstituted basement membrane (*12*) and placed in a Boyden blind well chemotaxis chamber. Conditioned medium (0.2 ml), obtained by incubating NIH 3T3 cells for 24 hours in serum-free medium, was placed in the lower compartment of the Boyden chamber. B16F10 melanoma cells were detached by the addition of 0.02% EDTA, suspended in Dulbecco's minimum essential medium (DMEM) containing 10% fetal calf serum, washed with DMEM containing 0.1% bovine serum albumin, and resuspended in this latter medium. Cells (3×10^5 per 0.8 ml) were placed in the upper compartment of the Boyden chamber with the peptides being tested. The concentrations of peptides in the upper compartment were 10, 50, and 100 μg/ml. After incubation for 5 hours at 37°C in 5% CO_2 and 95% air, the filters were fixed with methanol, stained with hematoxylin and eosin, and the cells migrating through the basement membrane were counted. In the absence of peptides, 25 cells migrated per field. Each sample was assayed in quadruplicate and the cells in at least five microscopic fields per filter were counted. Bar represents standard error of the mean.

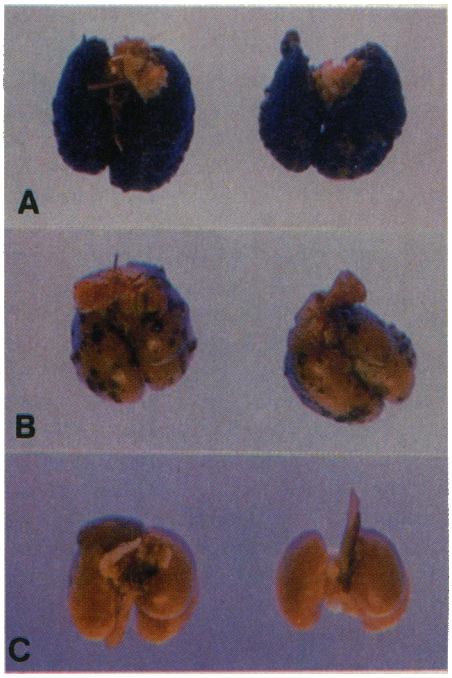

Fig. 3. Effect of peptide 11 and peptide 11–amide on lung tumor colonization. The lung tumor colonization assay was carried out as described (*13*). Peptide 11 (CDPGYIGSR) and peptide 11–amide were dissolved at 10 mg/ml in phosphate-buffered saline (PBS) [0.02M Na_2HPO_4 pH 7.4 and 0.15M NaCl] and were filter-sterilized. The suspension of B16F10 cells (5×10^5) in 0.1 ml of DMEM was mixed with 0.1 ml of the peptide, incubated for 5 minutes at room temperature, and then injected into the tail vein of syngeneic C57BL/6 female mice at 6 weeks of age. Each treatment and control group consisted of eight mice. Three weeks after the injections, the mice were killed and the number of pulmonary tumors on the surface of the lungs was counted. (**A**) Control lungs; (**B**) lungs from animals treated with 1 mg of peptide 11; (**C**) lungs from animals treated with 1 mg of peptide 11–amide.

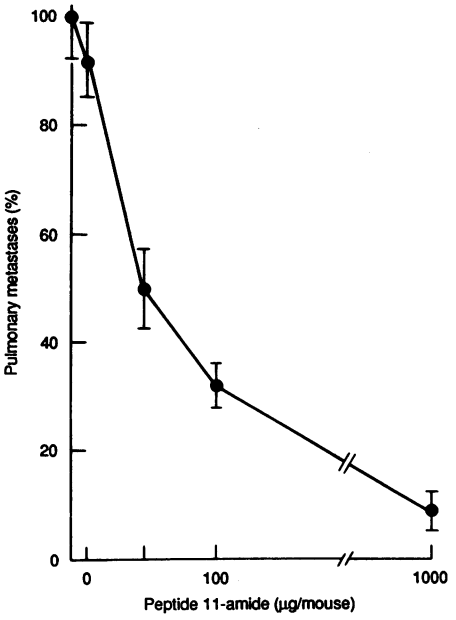

Fig. 4. Effect of peptide 11–amide on inhibition of lung tumor colonies. The assay was carried out as described (Fig. 3) except that various amounts of peptide (0.01 to 1 mg) were mixed with the cells and incubated for 5 minutes before injection. The average number of lung tumors in control mice (tumor cells plus PBS) was 51. Bar represents standard error of the mean (n = 8).

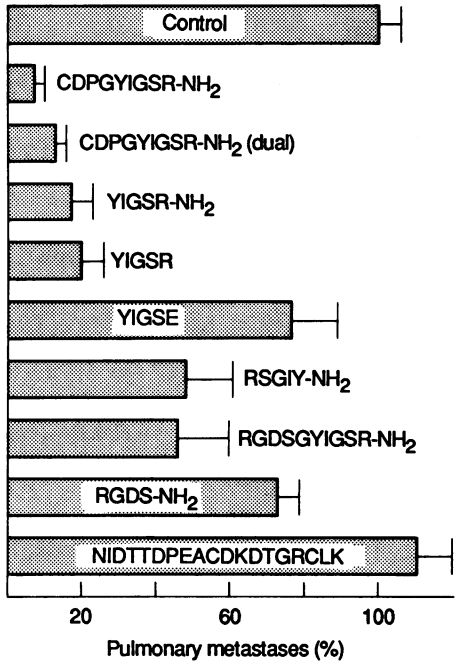

Fig. 5. Inhibitory effect of synthetic peptides on the formation of lung tumors. Peptides were solubilized at either 2 mg/ml (YIGSR) or 10 mg/ml (other peptides) in PBS and injected into mice as described (Fig. 3). Control mice received the same amount of cells and PBS without the peptide. In this group given dual injections of cells plus CDPGYIGSR-NH_2, mice were injected sequentially with melanoma cells via one tail vein and the peptide via the other. Each treatment and control group consisted of eight mice. Two weeks after the injection, mice were killed, lungs were removed, and the number of lung tumors was counted visually. At the time of autopsy, no extrapulmonary tumors were found. In the control mice (lacking the peptide), the average number of tumors was 60. Bar represents standard error of the mean.

laminin caused a fourfold increase in collagenase production (*18*). Taken together, we speculate that YIGSR may inhibit lung tumor colony formation by competing with laminin for the laminin receptor on tumor cells, thus blocking the binding of the cells to basement membranes. The fibronectin/vitronectin cell attachment peptide GRGDS also shows inhibitory activity in tumor cell colonization (*15*). The YIGSR sequence is specific to laminin whereas the RGDS sequence is present in over a hundred proteins (*20*). Since cells interact with both of these proteins via separate and specific receptors, it is likely that these peptides block tumor cell colonization by different mechanisms.

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Two Pairs of Recombination Signals Are Sufficient to Cause Immunoglobulin V-(D)-J Joining

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The minimum sequence requirements for antigen receptor V-(D)-J joining were studied by constructing recombination-substrates containing synthetic recombination signals and introducing them into a recombination-competent pre-B cell line. Two sets of heptamer (CACTGTG) and nonamer (GGTTTTTGT) sequences were shown to be sufficient to cause the V-(D)-J joining, if the 12– and 23–base pair spacer rule is satisfied. A point mutation in the heptamer sequence, or a change in the combination of the two spacer lengths, drastically reduced the recombination.

COMPLETE IMMUNOGLOBULIN (IG) and T cell receptor (TCR) variable region genes are generated by a somatic DNA rearrangement process, which assembles variable (V), diversity (D), and joining (J) gene segments during the differentiation of lymphocytes (*1–5*). Two blocks of sequences, a heptamer CACTGTG and a nonamer GGTTTTTGT are highly conserved adjacent to the germline V, D, and J segments (*6, 7*). The joining takes place between two pairs of recombination signal sequences (RSS's); one pair is separated by a 12–base pair (bp) spacer and the other by a 23-bp spacer (*8, 9*). Three approaches have been taken to study the molecular mechanism of V-(D)-J joining. One approach is the sequence analysis of the recombination region in the V, D, and J segments. Both Ig and TCR genes were shown to contain the heptamer and nonamer sequences separated by a spacer of constant length (Table 1). The second approach is the biochemical characterization of the enzymatic machinery responsible for V-(D)-J joining. It is assumed that at least three activities are needed in the V-(D)-J joining reaction: a DNA binding activity, an endonucleolytic activity, and ligase activity. Candidates for the endonucleolytic activity (*10–13*) were identified in pre-B cells. The third approach is the introduction of artificial recombination substrates with appropriate selectable markers into recombination-competent pre-B cells. Alt and Baltimore and their colleagues (*14, 15*) demonstrated that V-(D)-J joining could take place on exogenous genes. Lewis *et al.* (*14, 16*) identified V-J joining by DNA inversion between the exogenous V_κ and J_κ genes with a retroviral vector system (*17*). Blackwell and Alt (*15*) demonstrated that Ig heavy chain D-J joining occurred by DNA deletion on a plasmid vector introduced by DNA transformation. Yancopoulos *et al.* (*18*) found that even transfected TCR genes could rearrange in the pre-B line 38B9 (*15, 19*).

To define the minimum sequence requirements for the V-J type of recombination, we synthesized two oligonucleotides containing a pair of recombination signals, a heptamer and a nonamer; one pair was separated by a

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