

Laminin γ 1 chain peptide, C-16 (KAFDITYVRLKF), promotes migration, MMP-9 secretion, and pulmonary metastasis of B16–F10 mouse melanoma cells

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Laminin-1, a heterotrimer of α 1, β 1, and γ 1 chains specific to basement membrane, promotes cell adhesion and migration, proteinase secretion and metastases of tumour cells. Several active sites on the α 1 chain have been found to promote B16–F10 melanoma lung colonisation and here we have determined whether additional tumour promoting sites exist on the β 1 and γ 1 chains. Recently, we have identified novel cell adhesive peptides derived from laminin β 1 and γ 1 chains by systematic screening of synthetic peptides. Nine β 1 peptides and seven γ 1 peptides active for cell adhesion were tested for their effects on experimental pulmonary metastases of B16–F10 mouse melanoma cells *in vivo*. The most active adhesive peptide derived from the γ 1 chain globular domain, C-16 (KAFDITYVRLKF), significantly enhanced pulmonary metastases of B16–F10 cells, whereas no other peptides showed enhancement. C-16 also stimulated migration of B16–F10 cells in the Boyden chamber assay *in vitro*. Furthermore, C-16 significantly induced the production of MMP-9 from B16–F10 cells. These results suggest that this specific laminin γ 1 chain peptide has a metastasis-promoting activity and might be a new molecular target of anti-cancer treatment.

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Laminin-1 is part of a family of glycoproteins specific to basement membrane. It has multiple biological activities including promoting cell adhesion, migration, differentiation, neurite outgrowth and tumour cell malignancy (Timpl *et al*, 1979; Kleinman *et al*, 1985; Martin and Timpl, 1987; Timpl, 1989). Laminin-1 enhances the metastatic phenotype of tumour cells (Terranova *et al*, 1982, 1984; Barsky *et al*, 1984). In addition, laminin-1 induces production of collagenase IV (Turpeenniemi-Hujanen *et al*, 1986), urokinase-type plasminogen activator, and the 92-kDa matrix metalloproteinase (MMP-9) (Khan and Falcone, 1997) *in vitro*.

Several biologically active sites on mouse laminin-1 that affect tumour cells have been previously identified using synthetic peptides (Yamada and Kleinman, 1992). YIGSR on the β 1 chain promotes tumour cell adhesion and migration (Graf *et al*, 1987a,b; Iwamoto *et al*, 1988) and inhibits experimental pulmonary metastases of mouse melanoma cells and angiogenesis (Iwamoto *et al*, 1987; Sakamoto *et al*, 1991). IKVAV on the α 1 chain promotes cell adhesion, tumour growth, angiogenesis, collagenase IV activity by tumour cells, and experimental metastases as well as plasmino-

gen activator activation (Grant *et al*, 1989; Tashiro *et al*, 1989; Kanemoto *et al*, 1990; Stack *et al*, 1991; Nomizu *et al*, 1992).

Recently, we have systematically screened a large set of overlapping synthetic peptides covering the whole mouse laminin-1 for their cell adhesive activities. Five peptides from the G-domain of α 1 chain (Nomizu *et al*, 1995), 12 peptides from the γ 1 chain (Nomizu *et al*, 1997), 21 peptides from the short and long arms of α 1 chain (Nomizu *et al*, 1998), and 12 peptides from the β 1 chain (Nomizu *et al*, 2000) were found to have significant cell adhesive activity. In addition, several peptides from the laminin-1 have been identified to be active for angiogenesis (Malinda *et al*, 1999; Ponce *et al*, 1999), acinar formation of salivary gland (Hoffman *et al*, 1998), and neurite outgrowth (Powell *et al*, 2000).

We previously identified several laminin α 1 peptides that influence the metastatic activities of B16–F10 melanoma cells. AG-73 peptide from the α 1 G-domain causes liver metastases (Kim *et al*, 1998), A-13 peptide from the N-terminal globule promotes an increase in pulmonary metastases (Kuratomi *et al*, 1999), and the AG-73 peptide (LQVQLSIR) promotes increased lung colonies and liver metastases. Here we report effects of adhesive peptides from the β 1 and γ 1 chains on experimental pulmonary metastases of B16–F10 cells *in vivo*. We have screened other adhesive peptides from β 1 and γ 1 chains for metastatic activity. We found that one of the peptides derived from the short arm of γ 1 chain, C-16 (KAFDITYVRLKF), significantly enhanced pulmonary metastases of B16–F10 cells. This peptide also stimulated the migration of

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B16–F10 cells in the Boyden chamber assay. In addition, C-16 significantly induced production of MMP-9 by B16–F10 cells.

MATERIALS AND METHODS

Preparation of synthetic peptides

Cell adhesive peptides from the laminin β 1 and γ 1 chain and control peptides were synthesised and purified by high performance liquid chromatography as previously described (Nomizu *et al*, 1997). The purity and identity of the peptides were confirmed by an analytical HPLC and a Sciex API III triple quadrupole ion spray mass spectrometer.

Cells and culture

B16–F10 mouse melanoma cells (Fidler and Nicolson, 1976) (a gift of Dr IJ Fidler, Houston, TX, USA) were maintained in Dulbecco's modified Eagle's medium (DMEM; Life Technologies, Inc.) containing 10% foetal bovine serum (FBS; Hyclone, UT, USA), 100 units ml^{-1} penicillin, 100 $\mu\text{g ml}^{-1}$ streptomycin (Life Technologies, Inc.).

In vivo experimental pulmonary metastasis assay

For the *in vivo* experimental pulmonary metastasis assay, B16–F10 cells were detached by 0.02% EDTA in phosphate buffered saline (Versene, Life Technologies, Inc.) and suspended in DMEM (1 000 000 cells ml^{-1}). The cell suspension (0.2 ml) was injected into the tail veins of female C57BL6/N mice (9–12 weeks old). 0.2 mg of cell adhesion peptides (1 mg ml^{-1} in DMEM) was also intravenously injected within 10 min after the cell injection to exclude cell aggregations by mixture of peptide and cells. The mice were sacrificed 16 days after injection. The lungs were removed, and the number of visible colonies on the surface of the lungs was counted. When many colonies were formed and the number of the colonies could not be counted correctly because of the fusion of the colonies, the number was scored as 500. Five mice were used for each peptide. Duplicate experiments gave similar results.

All animals were maintained in filter top micro isolator cages and provided with sterile water and food under conditions complying with National Institute of Health (NIH) regulations. All manipulations of experimental animals were conducted in a laminar flow hood using strictly controlled procedures adhering to the UKCCCR Guidelines for the Welfare of Animals in Experimental Neoplasia to minimise stress or suffering (UKCCCR, 1998).

In vitro migration assay

Migration of B16–F10 cells through polycarbonate filters was assayed using 48 well chemotaxis chambers (modified Boyden chamber, Neuro Probe, MD, USA) as described previously (Kuratomi *et al*, 1999). The lower wells of the chamber were loaded with DMEM containing 0.1% bovine serum albumin (BSA, Sigma) (DMEM/BSA) and 100 $\mu\text{g ml}^{-1}$ of peptide. Versene-detached B16–F10 cells (50 000 cells per 50 μl in DMEM/BSA) were placed in the upper wells. After a 5 h incubation, cells on the lower surface of the filter were stained with Diff-Quik (Baxter, IL, USA), and counted under a microscope. Each peptide was tested in triplicate and each experiment was repeated at least twice.

Zymography

B16–F10 cells (2 500 000 cells) were plated onto 150 mm dishes with complete media. After 24 h, the media were replaced with serum-free DMEM containing various concentrations of peptides. The conditioned media were collected after a 16 h incubation at

37°C in 5% CO_2 and concentrated 50 times by using Centrprep 10 (Amicon). Equal aliquots of the conditioned media (20 μl per lane) were electrophoresed on 10% polyacrylamide gels containing 0.2% gelatin. The gels were washed with 10 mM Tris-HCl (pH 7.4) containing 2.5% Triton-X for 30 min, followed by two changes of 10 mM Tris-HCl (pH 7.4) for 30 min. After an overnight incubation in 50 mM Tris-HCl (pH 8.0) containing 5 mM CaCl_2 and 1 mM ZnCl_2 at room temperature, Coomassie blue was added to visualise the digested gelatin bands.

RESULTS

Effect of cell adhesive laminin β 1 and γ 1 chain peptides on experimental pulmonary metastasis of B16–F10 mouse melanoma cells

Nine peptides active for cell adhesion from the mouse laminin β 1 chain (Nomizu *et al*, 2000) and seven peptides from the γ 1 chain (Nomizu *et al*, 1997) were tested for their effects on *in vivo* experimental pulmonary metastases of B16–F10 mouse melanoma cells (Table 1). In control experiments (without peptides), the average of 163 metastatic colonies was formed on the surface of the lung within 16 days after B16–F10 cells were injected into the mouse tail vein (Table 2). Peptide C-16 (KAFDITYVRLKF) from the N-terminal globular domain of the γ 1 chain significantly enhanced the number of B16–F10 lung colonies (average of 482 colonies, Table 2 and Figure 1). No other peptides showed stimulation or inhibition of pulmonary metastases of B16–F10 cells (data not shown). A scrambled peptide of C-16, C-16T (FYAFKKITLVRD), slightly increased the number of B16–F10 lung colonies (average of 234 colonies with range from 90 to 345), but the difference was not significant (Table 2, Figure 1). These results suggested a sequence-specific enhancement of *in vivo* pulmonary metastases of B16–F10 cells by C-16.

In vitro cell migration

In order to study the mechanism of the enhancement of B16–F10 lung colonisation by C-16, we examined the activities of these 16 cell adhesive peptides for cell migration. Among the 16 cell adhesive peptides, 8 peptides (B-32, 34, 62, 133, 160; C-16, 64, 68) stimulated migration of B16–F10 cells, with C-16 having the

Table 1 Cell adhesive laminin β 1 and γ 1 chain peptides used in this study

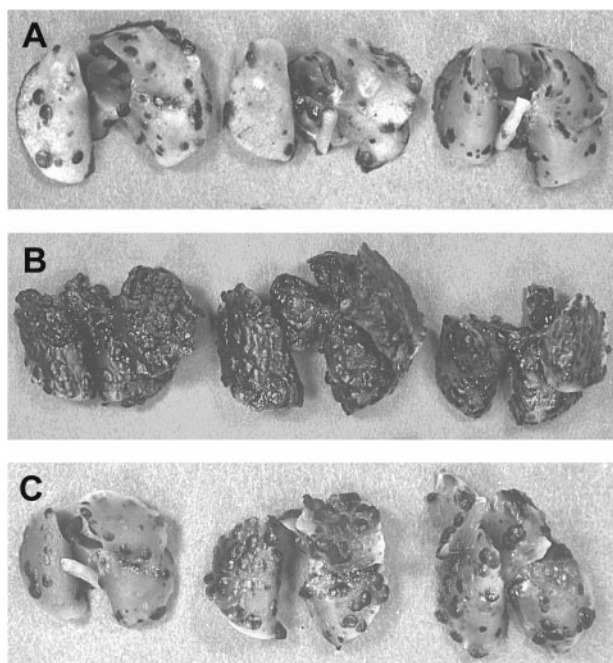
| Peptides | Sequences | Residues |
|-----------------------------------|----------------|-----------|
| <i>β1 chain</i> | | |
| B-20 | HLIMTFKTRPA | 114–125 |
| B-30 | RIQNLLKITNLR | 202–213 |
| B-31 | TNLRKIFVKLHT | 210–221 |
| B-32 | KLHTLGDNLLDS | 218–229 |
| B-34 | REKYYAVYDMV | 234–245 |
| B-54 | KRLVTGQR | 461–468 |
| B-62 | GPGVVVVEROYI | 544–555 |
| B-133 | DSITYYFQMSLE | 1298–1309 |
| B-160 | VILQQSAADIAR | 1538–1549 |
| <i>γ1 chain</i> | | |
| C-16 | KAFDITYVRLKF | 139–150 |
| C-28 | TDIRVTLNRLNTF | 245–257 |
| C-35 | LPIFNDRPWVRRAT | 318–330 |
| C-57 | APVKFLGNQVLSY | 559–571 |
| C-59 | SFSFRVDRRDTR | 576–587 |
| C-64 | SETTVKYIFRLHE | 615–627 |
| C-68 | TSIKIRGTYSER | 650–661 |

Cell adhesive peptides were identified by systematic screening of overlapping peptides covering the entire mouse laminin β 1 (Nomizu *et al*, 2000) and γ 1 chains (Nomizu *et al*, 1997).

Table 2 Effect of C-16 and C-16T on experimental pulmonary metastases by B16-F10 cells

| Treatment | Number of lung colonies per mouse | |
|-----------|-----------------------------------|---------|
| | Mean | Range |
| Control | 163 | 105–220 |
| C-16 | 481 | 445–500 |
| C-16T | 234 | 90–345 |

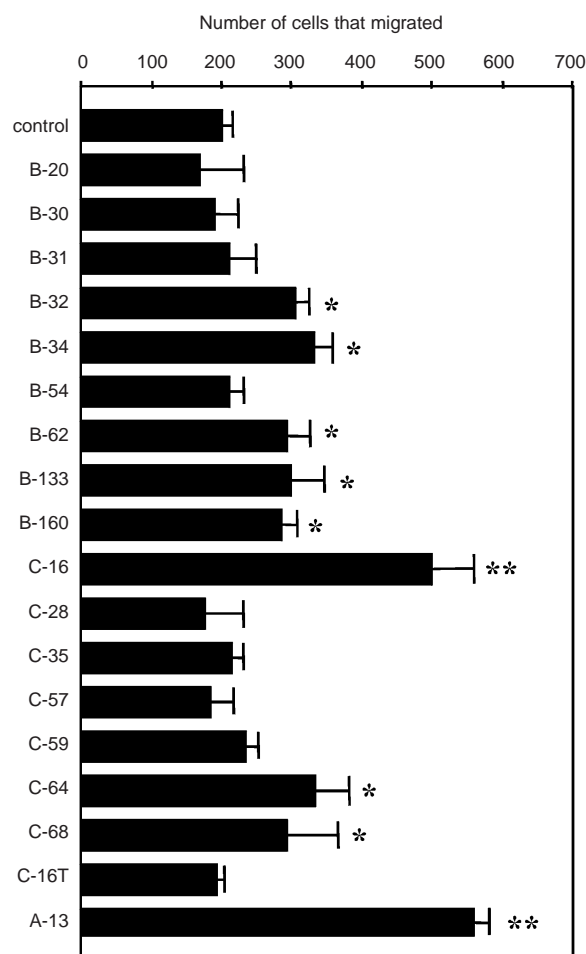
B16-F10 cells (200 000 cells per 0.2 ml) were intravenously injected, then either C-16 (0.2 mg per 0.2 ml) or C-16T (0.2 mg per 0.2 ml) was injected in the other tail vein within 10 min after cell injection. The number of visible colonies on the surface of the lungs was counted after 16 days. Data represent the average and the range of five mice. C-16 significantly increased the number of lung colonies ($P < 0.00001$), while C-16T showed no significant difference ($P < 0.28$). Duplicate experiments gave similar results. All other peptides listed in Table 1 did not increase lung colonisation.

**Figure 1** Appearance of the lung colonies formed by B16-F10 cells. Two hundred thousand cells per 0.2 ml were injected through the tail vein, then 0.2 mg per 0.2 ml of C-16 or the scrambled peptide of C-16, C-16T, was injected in the other tail vein within 10 min after the cell injection. Lungs were resected 16 days after the cell injection, then fixed in formalin, and photographed. C-16 (**B**) significantly promoted the formation of pulmonary metastases whereas C-16T (**C**) showed no enhancement as compared to control (**A**). Duplicate experiments gave similar results.

strongest enhancement activity (Figure 2). The migration-stimulatory activity of C-16 seemed to be comparable to that of peptide A-13 from the $\alpha 1$ chain which previously was shown to be highly active (Nomizu *et al*, 1998; Kuratomi *et al*, 1999). A control scrambled peptide, C-16T, showed no stimulatory activity of B16-F10 cell migration.

Gelatin zymography

Because matrix metalloproteinases (MMPs) have been implicated in the metastatic process of tumour cells, we measured MMP production from B16-F10 cells in the gelatin zymography assays (Figure 3). C-16 enhanced the production of MMP-9 (92 kDa gelatinase) in a dose-dependent manner. With $20 \mu\text{g ml}^{-1}$ of C-16, MMP-9 production was increased by approximately eight-fold over

**Figure 2** Effect of cell adhesive laminin $\beta 1$ and $\gamma 1$ chain peptides on B16-F10 cell migration. Migration of B16-F10 cells through the filters was assayed by using 48 well Boyden chambers. The upper chamber contained 50 000 cells and the lower chamber contained $100 \mu\text{g ml}^{-1}$ of peptide. After a 5 h incubation, the number of cells that migrated in the centre of each well was counted under the microscope. Data represent mean \pm s.d. of triplicate wells. Duplicate experiments gave similar results. * $P < 0.05$; ** $P < 0.01$.

that observed with the untreated control. MMP-2 production was not stimulated by C-16. No other cell adhesive peptides from the $\beta 1$ and $\gamma 1$ chains stimulated the production of MMPs (data not shown).

DISCUSSION

Laminin-1 has been shown to promote the metastatic activity of melanoma cells (Terranova *et al*, 1982, 1984). We previously reported that several peptide sequences including RQVFQVAYIII-KA (A-13), IKVAV, and LQVQLSIR (AG-73) from the laminin $\alpha 1$ chain have such activity (Kanemoto *et al*, 1990; Kim *et al*, 1998; Kuratomi *et al*, 1999). In this report, we identified a new site, C-16 (KAFDITYVRLKF), on the laminin $\gamma 1$ chain which promotes lung colonisation of B16-F10 cells in an experimental pulmonary metastasis model in mice.

C-16 enhanced B16-F10 colonization about three-fold compared with control. C-16 appears to be the only site on the $\gamma 1$ chain of laminin-1 active for lung colonisation and no site on the $\beta 1$ chain was found to have this activity. C-16-mediated metastasis promoting activity is comparable to that of A-13 and IKVAV. Since laminin-1 is a highly potent promoter of the malignant

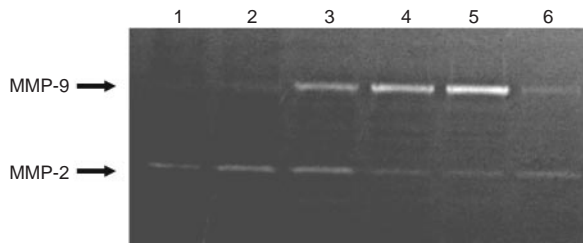


Figure 3 Effect of C-16 on activation of gelatinases. The conditioned media of B16–F10 cells incubated without peptide (lane 1) or with $2 \mu\text{g ml}^{-1}$ (lane 2), $5 \mu\text{g ml}^{-1}$ (lane 3), $10 \mu\text{g ml}^{-1}$ (lane 4), $20 \mu\text{g ml}^{-1}$ (lane 5) of C-16, or $20 \mu\text{g ml}^{-1}$ of C-16T (lane 6) were harvested and electrophoresed on 10% SDS gel containing 1% gelatin. C-16 stimulated the activity of MMP-9 (92-kDa gelatinase) in a dose-dependent manner, whereas C-16T showed no stimulation.

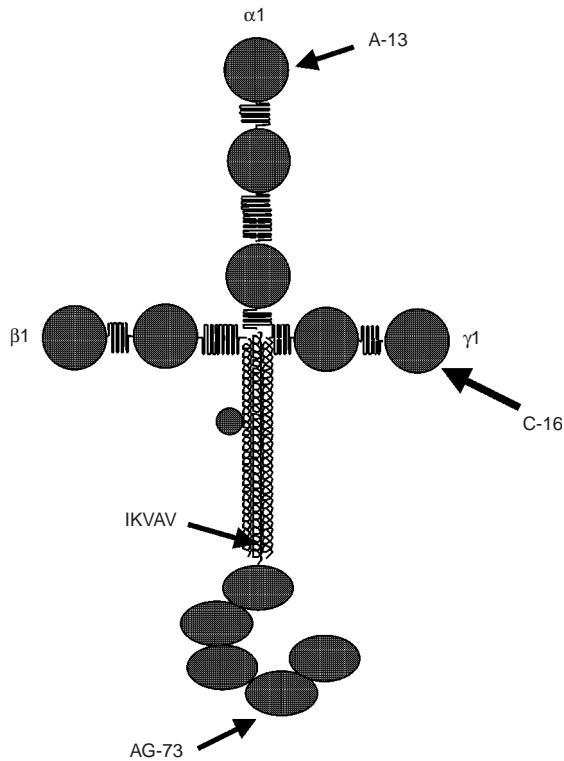


Figure 4 Novel metastasis-promoting peptides from the mouse laminin 1. AG-73 (Kim *et al*, 1998) and A-13 (Kuratomi *et al*, 1999) were found to promote experimental pulmonary metastases of B16–F10 cells. Previously, IKVAV was found to promote lung colonisation (Kanemoto *et al*, 1990). All peptides show stimulation of B16–F10 cell adhesion and migration activities. C-16, AG-73, and IKVAV stimulate 92-kDa gelatinase (MMP-9) activity of B16–F10 cells.

phenotype, it is not surprising that multiple sites for metastases have been identified.

The mechanism by which A-13 and C-16 increase lung colonisation is not known. Cells must adhere, migrate, invade (protease activity), proliferate, and generate blood supply in order to form

REFERENCES

Barsky SH, Rao CN, Williams JE, Liotta LA (1984) Laminin molecular domains which alter metastasis in a murine model. *J Clin Invest* **74**: 843–848

a growing metastatic lesion. A-13 and C-16 have been found to promote all of these activities except cell proliferation. The role of A-13 and C-16 in angiogenesis in the metastatic lesion has not been tested. We did not examine the vascularity of the lung lesions and it is unlikely that A-13 and C-16 persists in the circulation or tissue for very long after injection. These are small molecules which are cleared very rapidly from the circulation. We speculate that A-13 and C-16 may enhance lung metastases by increasing the invasion activity of the cells.

Laminin-1 stimulates cell migration (Kleinman *et al*, 1985) and laminin α 1 peptides with metastasis-promoting activity also have activity for the migration of B16–F10 cells (Kuratomi *et al*, 1999). We have shown here that five β 1 peptides and three γ 1 peptides stimulated migration of B16–F10 cells, with C-16 being most active and comparable to A-13. Thus, laminin-1 has multiple active sites for cell migration, which may function cooperatively.

Matrix metalloproteinases have been thought to be critical in tumour invasion and metastases as well as in angiogenesis (Liotta, 1986; Liotta *et al*, 1991; Kohn and Liotta, 1995). C-16 showed strong stimulation of MMP-9 production by B16–F10 cells. The IKVAV peptide from the laminin α 1 chain also induces MMP-1 and MMP-9 production from human monocyte/macrophage (Corcoran *et al*, 1995). AG-73 was also reported to stimulate MMP-9 activity of B16–F10 cells at the concentration of $50 \mu\text{g ml}^{-1}$ (Kim *et al*, 1998). C-16 is more potent in inducing MMP-9 production than AG-73. C-16 stimulated MMP-9 production nearly eight-fold at a concentration of $2 \mu\text{g ml}^{-1}$. This high activity of C-16 for MMP-9 production as well as for cell migration may explain its potent metastasis-promoting activity.

Recently, expression of the γ 2 chain of laminin-5, an epithelial cell-specific laminin, was predominantly detected at the invasive front of cancer cells of the colon, pancreas, stomach and oesophagus (Pyke *et al*, 1995; Soini *et al*, 1996; Sordat *et al*, 1998; Koshikawa *et al*, 1999; Yamamoto *et al*, 2001). The laminin γ 2 chain has been suggested to play a key role in the progression of human carcinomas. C-16 is located in the N-terminal globular domain of the laminin γ 1 chain, and this domain is deleted in the γ 2 chain. However, it is possible that the deleted N-terminal globular domain is co-expressed with laminin γ 2 chain by the proteolytic cleavage of the γ 2 chain and that the cleaved domain induces migration and metastases of tumour cells in human cancers. Indeed, Giannelli *et al* (1997) have reported that laminin-5 becomes active for promoting cell migration when cleaved by MMP-2. It is possible that these active fragments of laminin-1 promote cell migration when it is degraded similar to laminin-5.

Four novel active sites (IKVAV, AG-73, A-13 and C-16) on mouse laminin-1 have been identified for B16–F10 cell adhesion, migration, MMP secretion, and metastases in this report and in previous studies (Figure 4) (Kanemoto *et al*, 1990; Kim *et al*, 1998; Kuratomi *et al*, 1999). Interestingly, A-13 and C-16 are in homologous locations on their respective chains at the amino termini, binding to the same receptors and have similar activity (Ponce *et al*, 2001; and unpublished data). Of the 12 amino acids, three amino acids are identical and five are conserved. Preservation of sequences and activity suggest important functional sites. Degradation of laminin chains may also be important in exposure of these active sites. These active sites might be new molecular targets of anti-cancer treatments which prevent distant metastases of malignant tumours. Furthermore, specific antibodies to these sites and inhibitory peptides might lead to development of therapeutic agents for human malignant tumours.

Corcoran ML, Kibbey MC, Kleinman HK, Wahl LM (1995) Laminin SIKVAV peptide induction of monocyte/macrophage prostaglandin E2 and matrix metalloproteinases. *J Biol Chem* **270**: 10365–10368

- Fidler IJ, Nicolson GL (1976) Organ selectivity for implantation survival and growth of B16 melanoma variant tumor lines. *J Natl Cancer Inst* **57**: 1199–1202
- Giannelli G, Falk-Marzillier J, Schiraldi O, Stetler-Stevenson WG, Quaranta V (1997) Induction of cell migration by matrix metalloproteinase-2 cleavage of laminin-5. *Science* **277**: 225–228
- Graf J, Iwamoto Y, Sasaki M, Martin GR, Kleinman HK, Robey FA, Yamada Y (1987a) Identification of an amino acid sequence in laminin mediating cell attachment, chemotaxis, and receptor binding. *Cell* **48**: 989–996
- Graf J, Ogle RC, Robey FA, Sasaki M, Martin GR, Yamada Y, Kleinman HK (1987b) A pentapeptide from the laminin B1 chain mediates cell adhesion and binds the 67,000 laminin receptor. *Biochemistry* **26**: 6896–6900
- Grant DS, Tashiro K-I, Segui-Real B, Yamada Y, Martin GR, Kleinman HK (1989) Two different laminin domains mediate the differentiation of human endothelial cells into capillary-like structures in vitro. *Cell* **58**: 933–943
- Hoffman MP, Nomizu M, Roque E, Lee S, Jung DW, Yamada Y, Kleinman HK (1998) Laminin-1 and laminin-2 G-domain synthetic peptides bind syndecan-1 and are involved in acinar formation of a human submandibular gland cell line. *J Biol Chem* **273**: 28633–28641
- Iwamoto Y, Robey FA, Graf J, Sasaki M, Kleinman HK, Yamada Y, Martin GR (1987) YIGSR, a synthetic laminin pentapeptide, inhibits experimental metastasis formation. *Science* **238**: 1132–1134
- Iwamoto Y, Graf J, Sasaki M, Kleinman HK, Greatorex DR, Martin GR, Robey FA, Yamada Y (1988) Synthetic pentapeptide from the B1 chain of laminin promotes B16F10 melanoma cell migration. *J Cell Physiol* **134**: 287–291
- Kanemoto T, Reich R, Royce L, Greatorex D, Adler SH, Shiraishi N, Martin GR, Yamada Y, Kleinman HK (1990) Identification of an amino acid sequence from the laminin A chain that stimulates metastasis and collagenase IV production. *Proc Natl Acad Sci* **87**: 2279–2283
- Khan KMF, Falcone DJ (1997) Role of laminin in matrix induction of macrophage urokinase-type plasminogen activator and 92-kDa metalloproteinase expression. *J Biol Chem* **272**: 8270–8275
- Kim WH, Nomizu M, Song S-Y, Tanaka K, Kuratomi Y, Kleinman HK, Yamada Y (1998) Laminin α 1 chain sequence Leu-Gln-Val-Gln-Leu-Ser-Ile-Arg (LQVQLSIR) enhances murine melanoma cell metastasis. *Int J Cancer* **77**: 632–639
- Kleinman HK, Cannon FB, Laurie GW, Hassell JR, Aumailley M, Terranova VP, Martin GR, DuBois-Dalcq M (1985) Biological activities of laminin. *J Cell Biochem* **27**: 317–325
- Kohn EC, Liotta LA (1995) Molecular insights into cancer invasion: strategies for prevention and intervention. *Cancer Res* **55**: 1856–1862
- Koshikawa N, Moriyama K, Takamura H, Mizushima H, Nagashima Y, Yanoma S, Miyazaki K (1999) Overexpression of laminin γ 2 chain monomer in invading gastric carcinoma cells. *Cancer Res* **59**: 5596–5601
- Kuratomi Y, Nomizu M, Nielsen PK, Tanaka K, Song S-Y, Kleinman KH, Yamada Y (1999) Identification of metastasis-promoting sequences in the mouse laminin α 1 chain. *Exp Cell Res* **249**: 386–395
- Liotta LA (1986) Tumor invasion and metastases role of the extracellular matrix: Rhoads Memorial Award lecture. *Cancer Res* **46**: 1–7
- Liotta LA, Steeg PS, Stetler-Stevenson WG (1991) Cancer metastasis and angiogenesis: an imbalance of positive and negative regulation. *Cell* **64**: 327–336
- Malinda KM, Nomizu M, Chung M, Delgado M, Kuratomi Y, Yamada Y, Kleinman HK, Ponce ML (1999) Identification of laminin α 1 and β 1 chain peptides active for endothelial cell adhesion, tube formation and aortic sprouting. *FASEB J* **13**: 53–62
- Martin GR, Timpl R (1987) Laminin and other basement membrane components. *Annu Rev Cell Biol* **3**: 57–85
- Nomizu M, Utani A, Shiraishi N, Kibbey MC, Yamada Y, Roller PP (1992) The all-D-configuration segment containing the IKVAV sequence of laminin A chain has similar activities to the all-L-peptide in vitro and in vivo. *J Biol Chem* **267**: 14118–14121
- Nomizu M, Kim WH, Yamamura K, Utani A, Song S-Y, Otaka A, Roller PP, Kleinman HK, Yamada Y (1995) Identification of cell binding sites in the laminin α 1 chain carboxyl-terminal globular domain by systematic screening of synthetic peptides. *J Biol Chem* **270**: 20583–20590
- Nomizu M, Kuratomi Y, Song S-Y, Ponce ML, Hoffman MP, Powell SK, Miyoshi K, Otaka A, Kleinman HK, Yamada Y (1997) Identification of cell binding sequences in mouse laminin γ 1 chain by systematic peptide screening. *J Biol Chem* **272**: 32198–32205
- Nomizu M, Kuratomi Y, Malinda KM, Song S-Y, Miyoshi K, Otaka A, Powell SK, Hoffman MP, Kleinman HK, Yamada Y (1998) Cell binding sequences in laminin α 1 chain. *J Biol Chem* **273**: 32491–32499
- Nomizu M, Kuratomi Y, Ponce ML, Song S-Y, Miyoshi K, Otaka A, Powell SK, Hoffman MP, Kleinman HK, Yamada Y (2000) Cell adhesive sequences in mouse laminin β 1 chain. *Arch Biochem Biophys* **378**: 311–320
- Ponce ML, Nomizu M, Delgado MC, Kuratomi Y, Hoffman MP, Powell SK, Yamada Y, Kleinman HK, Malinda KM (1999) Identification of endothelial cell type specific sites on laminin γ 1 chain. *Circ Res* **84**: 688–694
- Ponce ML, Nomizu M, Kleinman HK (2001) An angiogenic laminin site and its antagonist bind through the α v β 3 and α 5 β 1 integrins. *FASEB J* **15**: 1389–1397
- Powell SK, Rao J, Roque E, Nomizu M, Kuratomi Y, Yamada Y, Kleinman HK (2000) Neural cell response to multiple novel sites on laminin-1. *J Neurosci Res* **61**: 302–312
- Pyke C, Salo S, Ralfkiaer E, Romer J, Dano K, Tryggvason K (1995) Laminin-5 is a marker of invading cancer cells in some human carcinomas and is coexpressed with the receptor for urokinase plasminogen activator in budding cancer cells in colon adenocarcinomas. *Cancer Res* **55**: 4132–4139
- Sakamoto N, Iwahana M, Tanaka NG, Osada Y (1991) Inhibition of angiogenesis and tumor growth by a synthetic laminin peptide, CDPGYIGSR-NH₂. *Cancer Res* **51**: 903–906
- Soini Y, Maatta M, Salo S, Tryggvason K, Autio-Harmanen H (1996) Expression of the laminin γ 2 chain in pancreatic adenocarcinoma. *J Pathol* **180**: 290–294
- Sordat I, Bosman FT, Dorta G, Rousselle P, Aberdam D, Blum AL, Sordat B (1998) Differential expression of laminin-5 subunits and integrin receptors in human colorectal neoplasia. *J Pathol* **185**: 44–52
- Stack S, Gray RD, Pizzo SV (1991) Modulation of plasminogen activation and type IV collagenase activity by a synthetic peptide derived from the laminin A chain. *Biochemistry* **30**: 2073–2077
- Tashiro K, Sephel GC, Weeks B, Sasaki M, Martin GR, Kleinman HK, Yamada Y (1989) A synthetic peptide containing the IKVAV sequence from the A chain of laminin mediates cell attachment, migration, and neurite outgrowth. *J Biol Chem* **264**: 16174–16182
- Terranova VP, Liotta LA, Russo RG, Martin GR (1982) Role of laminin in the attachment and metastasis of murine tumor cells. *Cancer Res* **46**: 2265–2269
- Terranova VP, Williams JE, Liotta LA, Martin GR (1984) Modulation of the metastatic activity of melanoma cells by laminin and fibronectin. *Science* **226**: 982–985
- Timpl R, Rohde H, Robey PG, Rennard SI, Foidart JM, Martin GR (1979) Laminin – a glycoprotein from basement membranes. *J Biol Chem* **254**: 9933–9937
- Timpl R (1989) Structure and biological activity of basement membrane proteins. *Eur J Biochem* **180**: 148–502
- Turpeenniemi-Hujanen T, Thorgeirsson UP, Rao CN, Liotta LA (1986) Laminin increases the release of type IV collagenase from malignant cells. *J Biol Chem* **261**: 1883–1889
- UKCCCR (1998) United Kingdom Co-ordinating Committee on Cancer Research (UKCCCR) Guidelines for the Welfare of Animals in Experimental Neoplasia 2nd edn. *Br J Cancer* **77**: 1–10
- Yamada Y, Kleinman HK (1992) Functional domains of cell adhesion molecules. *Curr Opin Cell Biol* **4**: 819–823
- Yamamoto H, Itoh F, Iku S, Hosokawa M, Imai K (2001) Expression of the γ (2) chain of laminin-5 at the invasive front is associated with recurrence and poor prognosis in human esophageal squamous cell carcinoma. *Clin Cancer Res* **7**: 896–900