The laminin-derived peptide YIGSR (Tyr–Ile–Gly–Ser–Arg) inhibits human pre-B leukaemic cell growth and dissemination to organs in SCID mice

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Summary

The YIGSR (Tyr–Ile–Gly–Ser–Arg) laminin β1 chain sequence has an inhibitory effect on tumour growth and the metastasis of melanoma and fibrosarcoma cells. In the present study, we investigated whether the multimeric YIGSR peptide (Ac-Y16) has an anti-proliferative effect and/or prevents the metastasis of human pre-B acute lymphoblastic leukaemia cells (NALM6) in severe combined immune deficient (SCID) mice. In vitro studies, Ac-Y16 significantly inhibited leukaemic cell colony formation and the invasion of NALM6 cells in a Matrigel-based assay. The tumour growth and leukaemic infiltration in peripheral tissues were also analysed in SCID mice 9 weeks after NALM6, Matrigel and Ac-Y16 were subcutaneously co-injected. The weight of the subcutaneous tumours was significantly suppressed by Ac-Y16 in a dose-dependent manner. Flow cytometry analysis showed that the leukaemic infiltration was significantly inhibited in all organs with 1.5–2.0 mg of Ac-Y16. Leukaemic infiltrations in the brain were inhibited with 0.5 mg of Ac-Y16, and those in brain and bone marrow were also inhibited with 1.0 mg of Ac-Y16. With Ac-S16, a control-scrambled peptide, the only significant inhibition of the leukaemic infiltration was observed in bone marrow at a much higher dose. These data suggest that the multimeric YIGSR peptide can inhibit the tumour growth and metastasis of leukaemic cells and may be useful as a potential therapeutic reagent for leukaemic infiltrations.

Keywords: laminin; multimeric YIGSR peptide; SCID mice; tumour metastasis; leukaemia cells

Tumour cell invasion and metastatic spread to peripheral organs have been found to proceed in three distinct stages: (1) the attachment of tumour cells to the basement membrane via cell surface receptors such as integrins and proteoglycans; (2) the degradation of basement membrane components, which is mediated by locally secreted enzymes; and (3) the migration of tumour cells into the digested matrix (Kramer et al, 1986; Albini et al, 1987; Aznavorian et al, 1993). Mature and neoplastic lymphoid cells circulate between blood and lymphoid organs by extravasation through both the endothelium and the basement membrane around capillaries, and then by migration along the extracellular matrix in the perivascular space (Segat et al, 1994). Matrigel, which is composed of basement membrane components including laminin, collagen type IV, proteoglycans and several growth factors, has been shown to promote the growth of leukaemic cells in xenograft models (Cavallo et al, 1991; Sterling-Levis et al, 1993). We have previously demonstrated the ability of Matrigel to promote tumour formation and dissemination to peripheral organs in severe combined immune deficient (SCID) mice using pre-B acute lymphoblastic leukaemia (ALL) cells (Ishii et al, 1995). Matrigel may provide a stromal-like support to leukaemic cells. The interaction between leukaemic cells and the matrix proteins via integrins or other cell surface receptors seems to be critical for the growth and dissemination of leukaemia in vivo.

Laminin-1, the major component of basement membrane and Matrigel, promotes cell adhesion, collagenase IV production, cell motility, and tumour growth and metastasis (Martin and Timpl, 1987; Timpl, 1989; Beck et al, 1990). Several active sites on laminin-1 have been identified using proteolytic fragments and synthetic peptides (Yamada and Kleinman, 1992). The peptide YIGSR (Tyr–Ile–Gly–Ser–Arg) comprised of residues 929–933 on the β1 chain has been found to inhibit tumour growth and metastasis (Graf et al, 1987; Iwamoto et al, 1987; Fridman et al, 1990). Both polymerized and polyethylene glycol-conjugated YIGSR peptides significantly enhanced the inhibitory effect of tumour metastasis (Murai et al, 1989; Kawasaki et al, 1991). In addition, when prepared as a multimeric peptide (Tam, 1988), YIGSR was shown to be a potent inhibitor of melanoma cell growth and metastasis (Nomizu et al, 1993). Multimeric YIGSR peptide was also shown to promote the apoptosis of fibrosarcoma cells, but not that of colon adenocarcinoma cells (Kim et al, 1994), suggesting cell-type specificity.

In the present study, we examined whether the multimeric YIGSR peptide has an anti-proliferative effect and/or prevents the metastasis of human pre-B ALL cells (NALM6) in SCID mice. We found that the multimeric YIGSR peptide significantly inhibited tumour growth and leukaemic infiltration in various organs.
MATERIALS AND METHODS

Synthesis of peptides

The multimeric YIGSR peptide (Ac-Y16), (CH₂CO-Tyr-Ile-Gly-Ser-Arg-Gly)₆-Lys₅-Lys₃-Lys₃-Lys₃-Lys₃-Lys₃-Lys₃-Gly ((Ac-YIGSR))₆-K₅K₄K₃K₂KG), was synthesized as described previously (Nomizu et al, 1993). The peptide has 16 copies of the YIGSR sequence and has a molecular weight of approximately 10 000. A scrambled multimeric peptide (Ac-S16), (Ac-GYSRG)₆-K₅K₄K₃K₂KG, was also synthesized as a control. The molecular weight of Ac-S16 is the same as that of AC-Y16. These peptides were purified by reverse-phase high-performance liquid chromatography. In this study, different amounts of Ac-Y16 (from 0.5 mg to 2.0 mg) were tested to examine the dose efficacy of the peptide. 1 ml of culture mixture containing leukaemic cells, Matrigel, serum albumin (Sigma Chemical Co., St Louis, MO, USA), and 5×10⁻³ mol l⁻¹ 2-mercaptoethanol (Sigma), was plated in a 35 mm dish (Becton Dickinson, Mountain View, CA, USA). Human and murine BM were incubated with 10 µl of fluorescein isothiocyanate (FITC)-conjugated OKB CALLA specific for CD10 (Ortho Diagnostic Systems, Raritan, NJ, USA) and 20 µl of PE-conjugated HD37 specific for CD19 (Dako, Glostrup, Denmark) to identify human leukaemic cells. Samples were then mixed gently, incubated at 4°C for 30 min and washed twice in phosphate-buffered saline (PBS). For the flow cytometric analysis, 2×10⁵ cells were analysed on a FACSscan (Becton Dickinson, Mountain View, CA, USA). Human and murine BM or peripheral blood cells were used for the gating of mononuclear cells. In each experiment, cells from non-transplanted mice were stained with the same antibodies, as a negative control. An isotype control antibody was also used. The percent infiltration of leukaemic cells was defined as the ratio of the number of CD10⁺CD19⁺-positive cells to that of all mononuclear cells in each organ.

RT-PCR

Total RNA was extracted from tumour and organ homogenates (Ishii et al, 1995). cDNA was prepared by RT at 37°C for 60 min in a 50 µl mixture containing 5 µg RNA, 1 µg oligo-dT and 100 units of MMLV reverse transcriptase (Life Technology, Rockville, MD, USA). The integrity of RNA and cDNA was confirmed by the generation of a β-actin PCR product (228 bp) with primers which hybridize with both human and murine β-actin (actin-MH): 5'CTACAATGAGCTGCGTGTGG-3' and 5'TAGATGGCACCAGTGTGGGT3' (Nakajima-Iijima et al, 1985). Primers specific for human β-actin (actin-H) were also prepared to detect human leukaemic cells in mouse organs. These primers were 5'GGCAGCGTCTGCTTCCAG-3' and 5'CATTTTGCTGGTGCCAGG-3' (Nakajima-Iijima et al, 1985). The expected size of the PCR product was 295 bp. The PCR was performed with cDNA in a total volume of 50 µl, containing 1× PCR buffer, 200 µM
dNTPs, 0.5 μg each of the 5′ and 3′ primers and 2.5 units of Taq polymerase (Perkin-Elmer, Norwalk, CT, USA). The amplification profile involved 30 cycles of denaturation at 94°C for 30 s, primer annealing at 53°C for 30 s and extension at 72°C for 1 min. The PCR products were resolved by electrophoresis in 2% agarose gels, stained with ethidium bromide and photographed. The reproducibility of the PCR analysis was confirmed by several independent experiments.

Apoptosis assays
NALM6 cells (4 × 10⁶ ml⁻¹) were co-incubated with 2–50 μg of Ac-Y16 or Ac-S16 for 12 h at 37°C. After staining leukemic cells with 10 μl of FITC-conjugated annexin V and 5 μl of propidium iodide (MEBCYTO-Apoptosis Kit, MBL, Japan) for 15 min, the number of apoptotic cells was measured by flow cytometry (van Engeland et al., 1998). We also measured the mitotic index of leukemic cells and the number of apoptotic cells in the s.c. tumours by histological examination and/or TUNEL assays (ApoptaqPlus, Oncor, Gaithersburg, MD, USA).

Statistical analysis
Differences in the colony formation and invasion of leukaemic cells in vitro between the test concentration groups were compared by the Kruskal–Wallis test. The tumour weight and the infiltration of leukaemic cells in mice with or without Ac-Y16 or Ac-S16 were also compared by the Kruskal–Wallis test. A multiple comparison test was performed using the method of Bonferroni adjustment when the Kruskal–Wallis test showed P < 0.05 (Alt, 1982).

RESULTS
Effect of Ac-Y16 on the colony formation and invasion of leukaemic cells
We examined the effect of Ac-Y16 on the colony formation of leukaemic cells in vitro. NALM6 cells were incubated with Matrigel and 0.5–2.0 mg of Ac-Y16 or 1.5 mg of Ac-S16, and the number of leukaemic colonies was measured after 7 days of incubation. As shown in Figure 1A, the number of colonies was decreased with increasing amounts of Ac-Y16. A significant difference was observed between test concentration groups (P < 0.01). Ac-S16, the control-scrambled peptide, did not inhibit the colony formation of NALM6 cells. Thus, the Ac-Y16 peptide inhibited leukaemic cell growth in vitro.

The in vitro effect of Ac-Y16 on the invasion of leukaemic cells was also examined (Figure 1B). Approximately 1.25–1.50% of the leukaemic cells migrated through a Matrigel-coated membrane. With increasing amounts of Ac-Y16, the percentage of leukaemic cells that migrated into lower compartment was significantly decreased (P < 0.05). Ac-S16 showed little effect on the cell migration. These data suggest that laminin plays an important role in leukaemic cell invasion in vitro.

To examine whether Ac-Y16 induces apoptosis of leukaemic cells, NALM6 cells were co-cultured with 2–50 μg of Ac-Y16 or Ac-S16 and the number of apoptotic cells was measured by flow cytometry. The percentage of apoptotic cells was less than 3% in control leukemic cells, whereas in cells treated with Ac-Y16, 10.8% at 2 μg ml⁻¹, 10.3% at 10 μg ml⁻¹ and 33.1% at 50 μg ml⁻¹ of Ac-Y16 were apoptotic after 12 h of treatment. Control Ac-S16 showed less apoptosis than Ac-Y16, i.e. 8.8% at 2 μg ml⁻¹, 8.1% at 10 μg ml⁻¹ and 13.5% at 50 μg ml⁻¹.

Effects of the multimeric YIGSR (Ac-Y16) peptide on tumour growth and the dissemination of leukaemic cells in SCID mice
Human pre-B leukaemia NALM6 cells (2 × 10⁶) and Matrigel (2 mg) were injected s.c. in SCID mice with or without the multimeric YIGSR peptide (Ac-Y16). As a control, Ac-S16 was also injected with Matrigel and NALM6 cells. After 9 weeks, the growth of the primary tumour and the infiltration of leukaemic cells into the spleen, liver, lung, kidney, brain and BM were...
analysed. The weight of the s.c. tumours differed significantly in the mice with or without Ac-Y16 or Ac-S16 ($P = 0.0004$) (Figure 2). No tumour formation was observed in mice when 1.5 or 2.0 mg of Ac-Y16 was coinjected. One milligram of Ac-Y16 significantly inhibited tumour growth ($P < 0.01$) by more than 90%. In contrast, when 1.5 mg of Ac-S16 were co-injected with leukaemic cells, a lesser inhibitory effect on tumour growth was observed. However, these differences were not significant (Figure 2). To examine whether the inhibition by Ac-Y16 of tumour growth is due to the inhibition of cell proliferation, to cell death by apoptosis, or both, we measured the mitotic index of leukaemic cells and the number of apoptotic cells of the s.c. tumours. The mitotic index and the number of apoptotic cells were not significantly different in the s.c. tumours with and without Ac-Y16 or Ac-S16 (data not shown). These findings were confirmed by the staining with MIB-1 monoclonal antibodies to detect mitotic cells.

The infiltration of leukaemic cells in peripheral organs was assessed in a flow cytometry analysis. The flow cytometric profiles of representative samples obtained from untreated control mice and those with leukaemic cells and Matrigel are given in Figure 3. All of the organs from untreated mice showed no or very low reactivity with both the CD10 and CD19 antibodies, whereas high numbers of CD10$^+$CD19$^+$ cells were detected in organs from mice with leukaemic cells and Matrigel. Figure 4 shows the percent infiltration of leukaemic cells in all organs from six different
Figure 4 Infiltration of leukaemic cells in peripheral organs. The leukaemic infiltration, defined as the per cent of CD10+CD19+ human cells in each organ, was measured by flow cytometry and plotted. Each horizontal bar shows the mean of the per cent infiltration of leukaemic cells in mice. Lane 1, mice with Matrigel \( (n=8) \); lane 2, mice with Matrigel and 0.5 mg Ac-Y16 \( (n=8) \); lane 3, mice with Matrigel and 1.0 mg Ac-Y16 \( (n=8) \); lane 4, mice with Matrigel and 1.5 mg Ac-Y16 \( (n=8) \); lane 5, mice with Matrigel and 2.0 mg AC-Y16 \( (n=5) \); lane 6, mice with Matrigel and 1.5 mg Ac-S16 \( (n=5) \).
groups of mice. High levels of leukemic infiltration were observed in the spleen, liver, lung, kidney, brain and BM of all mice with Matrigel alone. The infiltration of leukemic cells in the mice with and without Ac-Y16 or Ac-S16 was significantly different in all organs: \( P < 0.0075 \) in spleen, \( P = 0.0001 \) in liver, \( P = 0.0001 \) in lung, \( P = 0.0018 \) in kidney, \( P < 0.0001 \) in brain and \( P < 0.0001 \) in BM. Two mg of Ac-Y16 completely suppressed the infiltration of leukemic cells in all organs. At 1.5 mg of Ac-Y16, the leukemic infiltration was significantly inhibited in the spleen (\( P < 0.05 \)) and other organs (\( P < 0.01 \)), which is also shown in Figure 3. Of all the organs examined, the brain was the most sensitive to the presence of the test peptide. Only a low infiltration of leukemic cells was observed in the brain of mice treated with Ac-Y16 at 0.5 mg or at 1.0 mg. In contrast, with 1.5 mg of Ac-S16, a significant inhibition of leukemic infiltration was observed only in BM (\( P < 0.01 \)).

The dissemination of leukemia cells was also assessed by the RT-PCR analysis of human \( \beta \)-actin mRNA expression in the spleen, brain and BM (Figure 5). Human \( \beta \)-actin mRNA was detected in the spleen, brain and BM of mice treated with Matrigel alone. In the mice treated with 1.0 mg of Ac-Y16, the expression of human \( \beta \)-actin mRNA was observed in only the spleen and BM; the brain showed undetectable levels of human \( \beta \)-actin mRNA. Mice treated with 1.5 mg of Ac-Y16 showed undetectable mRNA of \( \beta \)-actin in all organs. In contrast, the human \( \beta \)-actin mRNA was detected in mice with 1.5 mg of Ac-S16. The BM in mice treated with Ac-S16, which showed only a small infiltration of leukemic cells by flow cytometry, also expressed human \( \beta \)-actin by RT-PCR. Thus, Ac-Y16 inhibited the tumour growth and dissemination of leukemic cells into peripheral organs in a dose-dependent manner.

DISCUSSION

The Tyr–Ile–Gly–Ser–Arg (YIGSR) sequence derived from the laminin \( \beta 1 \) chain has been shown to inhibit tumour growth and metastasis (Graf et al, 1987; Iwamoto et al, 1987; Saiki et al, 1989; Fridman et al, 1990). It was reported that the multimeric YIGSR polypeptide greatly enhanced the inhibition of tumour growth and metastasis (Nomizu et al, 1993). Although the mechanisms of this effect of YIGSR are still not clear, recent results have suggested that apoptosis may play a role in the anti-metastatic and anti-tumour effects associated with multimeric YIGSR peptide in HT-1080 human fibrosarcoma cells (Kim et al, 1994). However, cell type-specific apoptosis by YIGSR has not been demonstrated (Kim et al, 1994). It was also reported that YIGSR reduces angiogenesis (Sakamoto et al, 1991; Iwamoto et al, 1996).

The interaction between Matrigel and leukemic cells can also facilitate a proliferative response (Sterling-Levis et al, 1993; Ishii et al, 1995; Yan et al, 1996). As shown in a previous report (Blase et al, 1996), NALM6 expressed high levels of VLA-\( \alpha 3 \), \( \alpha 4 \), \( \alpha 5 \) and \( \alpha 6 \), and VLA-\( \beta 1 \), VLA-\( \alpha 6 \), which is usually expressed on pre-B leukemic cells, interacts with laminin (Hynes, 1992). In fact, NALM6 cells mainly adhere to laminin, and this binding is significantly reduced by the \( \beta 1 \) and \( \alpha 6 \) monoclonal antibodies (Blase et al, 1996). We also found that Ac-Y16 has similar activity for NALM6 cell attachment (data not shown). However, the binding site of laminin-1 for VLA-\( \alpha 3 \) and \( \alpha 6 \) integrins is the C-terminal portion (Hall et al, 1990; Tomasselli et al, 1990; Sonnenberg et al, 1991), while YIGSR has been shown to recognize 36-kDa, 38-kDa and 67-kDa cell surface proteins (Graf et al, 1987; Clement et al, 1990) and \( \alpha 4 \beta 1 \) integrin (Maeda et al, 1994). Taken together, these findings indicate that YIGSR may inhibit tumour formation and leukemic inhibition by competing with laminin for these laminin receptors and/or integrins on leukemic cells, thus blocking the binding of the cells to basement membrane (Iwamoto et al, 1987).

In our study, the growth and dissemination of leukemic cells were inhibited by the multimeric YIGSR peptide in vivo and in vitro. The precise mechanism of the drug-provoked tumour inhibition is unclear. Although the direct toxicity of YIGSR peptide for leukemic cells cannot be completely ruled out, Ac-Y16 reduced tumour growth at 0.5–1.0 mg in vitro and selectively inhibited the dissemination of leukemic cells in vivo. Previous data also suggested that Ac-Y16 is not cytotoxic in vivo (Iwamoto et al, 1996). In the present study, the high dose (1.5–2.0 mg) of Ac-Y16 clearly inhibited the tumour formation and leukemic infiltration in all peripheral organs, compared with the same dose of Ac-S16, a scrambled multimeric peptide, which showed only a weak inhibitory effect on leukemic infiltration. Apoptosis of NALM6 cells were induced by Ac-Y16 in cultures, whereas the number of apoptotic cells in the s.c. tumours was not increased by Ac-Y16. Although this discrepancy is not clear, it is possible that the sensitivity of the apoptosis assays may be different between cell cultures and in vivo. Another possibility is that Ac-Y16-mediated apoptosis may occur in much earlier stages after the inoculation of NALM6 cells with Ac-Y16 in SCID mice. Our apoptosis assays were performed at 12 h after the incubation and at this late stage apoptotic cells may not be present and could not be detected. Alternatively, Ac-Y16 may be more potent in inhibiting tumour cell proliferation or inducing necrosis of tumour cells than apoptosis in vivo.

In the previous study, the proliferation of HT-1080 cells was markedly decreased by Ac-Y16 at 60–100 \( \mu \)g ml\(^{-1}\), while only a small effect was observed at 30 \( \mu \)g ml\(^{-1}\) (Kim et al, 1994). Proliferation of SW480 cells was reduced at 100 \( \mu \)g ml\(^{-1}\), but had no effect at 30 \( \mu \)g ml\(^{-1}\) (Kim et al, 1994). In our study, the colony formation of leukemic cells was partially suppressed by Ac-Y16 at 0.5–2.0 mg per \( 10^6 \) cells (5–20 \( \mu \)g ml\(^{-1}\)). Although assays used for these two studies are different, their data suggest that the inhibitory activity of Ac-Y16 varies with different cell types.

Leukemic cells usually spread from bone marrow or the tumour burden to peripheral organs as overt leukaemia. In order to
disseminate, leukemic cells enter the circulatory system by crossing the endothelium and the basement membrane. The multimeric YIGSR peptide may inhibit the spreading of leukemic cells to the vascular endothelium, by blocking leukemic cell binding to laminin. Only one leukemic cell line was used in the present study; further analyses is necessary to examine the inhibitory activity of Ac-Y16 for primary leukemic cells from patients.

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REFERENCES

Albini A, Iwamoto Y, Kleinman HK, Martin GR, Aronson SA, Kozlowski JM and McEwan RN (1987) A rapid in vitro assay for quantitating the invasion potential of tumor cells. Cancer Res 47: 3239–3245

Alt FB (1982) Bonferroni Inequalities and Intervals. Encyclopedia of Statistical Sciences. Vol. 1, pp. 294–300. John Wiley & Sons: New York

Aznarvoorian S, Murphy AN, Sterlet-Stevenson WG and Liotta LA (1993) Molecular aspects of tumor cell invasion and metastasis. Cancer 71: 1386–1393

Beck K, Hunter Land Engel J (1990) Structure and function of laminin: anatomy of multidomain glycoprotein. FASEB J 4: 148–160

Blase L, Merling A, Engelman S, Moller P and Schwartz-Albiez R (1996) Characterization of cell surface-expressed proteochondroitin sulfate of pre-B Nalm-6 cells and its possible role in laminin adhesion. Leukemia 10: 1001–1005

Cavollo F, Riccardi C, Forini M, Pericle F, Bosco MC, Giovarcei M, Soleti A and Forni G (1991) Growth and dissemination of human malignant lymphoblasts in immuno suppressed nu/nu mice. Nat Immun Cell Growth Regul 10: 256–264

Clement B, Segui-Real B, Savagner P, Kleinman HK and Yamada Y (1990) Hepatocyte attachment to laminin is mediated through multiple receptors. J Cell Biol 110: 185–192

Fridman R, Giaccone G, Kanemoto T, Martin GR, Kleinman HK and Yamada Y (1993) Multimeric forms of the laminin sequence YIGSR peptide may inhibit the spreading of leukaemic cells. Eur J Biochem 54: 57–65

Ishii E, Greaves A, Grunberger T, Freedman MH and Letarte M (1995) Tumor cells. American Society for Microbiology. Washington, DC

Irvin JD, Myers DE and Gunther R (1992) In vivo efficacy of B43 (anti-CD19)-pokeweed antiviral protein immunotoxin against human pre-B cell leukemia cell line. J Biol Macromol 11: 97–99

Kawasaki K, Namikawa M, Murakami T, Mizuta T, Iwai Y, Hama T and Mayumi T (1991) Amino acids and peptides. XIV. Laminin related peptides and their inhibitory effect on experimental metastasis formation. Biochem Biophys Res Commun 174: 1159–1162

Kim WH, Schnaper W, Nomizu M, Yamada Y and Kleinman HK (1994) Apotosis in human fibrosarcoma cells is induced by a multimeric synthetic Tyr-Ile-Gly-Ser-Arg (YIGSR)-containing polypeptide from laminin. Cancer Res 54: 5005–5010

Kramer RH, Bensch KG and Wong J (1986) Invasion of reconstituted basement membrane matrix by metastatic human tumor cells. Cancer Res 46: 1980–1989

Maeda T, Titani K and Sekiguchi K (1994) Cell-adhesive activity and receptor-binding specificity of the laminin-derived YIGSR sequence grafted onto Sphyvlococcal protein. A J Biochem (Tokyo) 115: 182–189

Martin GR and Timpf R (1987) Laminin and other basement membrane components. Annu Rev Cell Biol 3: 57–85

Murata J, Saiki I, Azuma I and Nishi N (1989) Inhibitory effect of a synthetic polypeptide, polypeptide of Tyr-Ile-Gly -Ser-Arg, on the metastatic formation of malignant tumor cells. J Biochem Macromol 11: 97–99

Nakahata T and Ogawa M (1982) Hemopoietic colony-forming cells in umbilical cord blood with extensive capability to generate mono- and multipotent hemopoietic progenitors. J Clin Invest 70: 1324–1338

Nakajima-Iijima S, Hamada H, Reddy P and Kakunaga T (1985) Molecular structure of the human cytoplasmic β-actin gene: interspecies homology of sequences in the introns. Proc Natl Acad Sci USA 82: 6133–6137

Nakamura K, Yamamura K, Kleinman HK and Yamada Y (1993) Multimeric forms of Tyr-Ile-Gly-Ser-Arg (YIGSR) peptide enhance the inhibition of tumor growth and metastasis. Cancer Res 53: 3459–3461

Iwamoto Y, Iwahana M, Tanaka NG and Osada Y (1991) Inhibition of angiogenesis and tumor growth by a synthetic laminin peptide. Cancer Res 51: 903–906

Segat D, Pucillo C, Marotta G, Perris R and Colombatti A (1994) Differential attachment of human neoplastic B cells to purified extracellular matrix molecules. Blood 83: 1586–1594

Sturzenegger A, Geahlen KR, Aumailley M and Timpl R (1991) Isolation of α1β1 integrins from mouse platelets and adherent cells by affinity chromatography on mouse laminin fragment E8 and human laminin pep fragment. Exp Cell Res 197: 234–244

Sterling-Levis K, White L, Trickett AE, Gramacho C, Pittman SM and Tobias V (1993) Heterotransplantation of early B-lineage acute lymphoblastic leukemia using a solubilized attachment matrix (matrigel). Cancer Res 53: 1222–1225

Tan FM (1988) Synthetic peptide vaccine design: synthesis and properties of a high-density multiple antigenic peptide system. Proc Natl Acad Sci USA 85: 5409–5413

Timpl R (1989) Structure and biological activity of basement membrane proteins. Eur J Biochem 180: 148–202

Tomasselli KJ, Hall DE, Fler LA, Geahlen KR, Turner DC, Carbonetto S, Reichardt LF (1990) A neuronal cell line (PC12) expresses two a1β1 class integrins – α1β1 and αβ1 – that recognize different neurite outgrowth-promoting domains in laminin. Neuron 5: 651–662

Uckun FM, Manivel C, Arthur D, Chelstrom LM, Finnegan D, Tuel-Ahlgren L, Ivins JD, Myers DE and Gunther R (1992) In vivo efficacy of B43 (anti-CD19)-pokeweed antiviral protein immunotoxin against human pre-B cell leukemia cell line. Blood 80: 2201–2214

van Engeland M, Nieland LJ, Ramaekers FC, Schutte B, Reutelingsperger CP (1998) Annexin V-affinity assay: a review on an apoptosis detection system based on phosphotidylserine exposure. Cytometry 31: 1–9

Yamada Y and Kleinman HK (1992) Functional domains of cell adhesion molecules. Curr Opin Cell Biol 4: 819–823

Yamada Y, Zollom OA, McGreen T, Denning D, Fernandez J, Jagiello C, Collins N, Steinheiz P and O’Reilly RJ (1996) Growth pattern and clinical correlation of subcutaneously inoculated human primary acute leukemias in severe combined immunodeficiency mice. Blood 88: 3137–3146

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