# Identification of binding peptides of the ADAM15 disintegrin domain using phage display

Jing Wu[1,2], Min-Chen Wu[1,2], Lian-Fen Zhang[1,2], Jian-Yong Lei[1,2], Lei Feng[1,2] and Jian Jin[1,2,*]

[1]*School of Medicine and Pharmaceutics, Jiangnan University, Wuxi, Jiangsu 214122, China*

[2]*The Key Laboratory of Industrial Biotechnology, Ministry of Education, Jiangnan University, Wuxi, Jiangsu 214 122, China*

**Corresponding author (Fax, +86-510-85918219; Email, jinjian31@hotmail.com)*

ADAM15 plays an important role in tumour development by interacting with integrins. In this study, we investigated the target peptides of the ADAM15 disintegrin domain. First, we successfully produced the recombinant human ADAM15 disintegrin domain (RADD) that could inhibit melanoma cell adhesion by using *Escherichia coli*. Second, four specific binding peptides (peptides A, B, C, and D) were selected using a phage display 12-mer peptide library. The screening protocol involved 4 rounds of positive panning on RADD and 2 rounds of subtractive selection with streptavidin. By using the BLAST software and a relevant protein database, integrin $\alpha_v\beta_3$ was found to be homologous to peptide A. Synthetic peptide A had a highly inhibitory effect on RADD–integrin $\alpha_v\beta_3$ binding. The results demonstrate the potential application of short peptides for disrupting high-affinity ADAM–integrin interactions.

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## 1. Introduction

ADAMs (A Disintegrin and Metalloproteinases) with multiple functional domains are a family of membrane-anchored glycoproteins involved in proteolysis, cell adhesion, cell fusion and intracellular signalling (Arribas *et al.* 2006; Mochizuki and Okada 2007). The disintegrin domains of ADAMs are believed to interact with the integrin superfamily and perform various biological functions. The specificity and affinity of ADAMs toward distinct integrins are determined by a tripeptide integrin-binding motif. ADAM15 is unique in that it contains the integrin-binding motif Arg-Gly-Asp (RGD) within its disintegrin domain (Kratzschmar *et al.* 1996; Stone *et al.* 1999).

Interaction of ADAM15 with integrins has been shown to be important in several biological processes. For example, ADAM15 serves as a binding partner of integrin $\alpha_v\beta_3$ to decrease the adhesion and motility of ovarian cancer cells (Beck *et al.* 2005). The recombinant ADAM15 disintegrin domain expressed in *Escherichia coli* can inhibit airway smooth muscle cell (ASMC) adhesion and migration by interacting with subunits of $\beta_1$-integrins (Lu *et al.* 2007). Further, yeast-expressed ADAM15 disintegrin-like domains could inhibit the binding of $\alpha_{IIb}\beta_3$ as well as $\alpha_v\beta_3$ to their biological ligands, and these domains also had an inhibitory effect on human platelet aggregation (Jeon *et al.* 2007). Moreover, results from mutation experiments suggested that most ADAM15–integrin interactions were RGD-dependent

**Keywords.** Inhibitory effect; integrin $\alpha_v\beta_3$; interaction; phage display; recombinant ADAM15 disintegrin domain; target peptide

Abbreviations used: ADAM, A Disintegrin And Metalloproteinase; ASMC, airway smooth muscle cell; b-RADD, biotinylated RADD; BSA, bovine serum albumin; DMEM, Dulbecco modified Eagle medium; EGF, epidermal growth factor; ELISA, enzyme-linked immunosorbent assay; GST, glutathione *S*-transferase; HBSS, Hanks balanced salt solution; HMEC, human microvascular endothelial cells; HPLC, high-performance liquid chromatography; HRP, horseradish peroxidase; IL, interleukin; IPTG, isopropyl-1-thio-*β*-D-galactopyranoside; MALDI-TOF, matrix-assisted laser desorption ionisation time of flight; OPD, o-phenylenediamine dihydrochloride; PBS, phosphate buffered saline; RADD, recombinant human ADAM15 disintegrin domain; RGD, Arg-Gly-Asp; TBST, Tris-buffered saline Tween-20

(Zhang *et al.* 1998). However, the target peptide of ADAM15 on integrins or any other binding protein remains unknown.

Phage display is a molecular biology tool by which target-binding peptide sequences can be identified. In this method, facile selection and screening of large and diverse peptide libraries allows the identification of peptides that bind to immobilised target proteins (Azzazy and Highsmith 2002). Phage clones with a high affinity for the target protein are identified by DNA sequencing, and their corresponding amino acid sequences are determined. Phage display has been successfully used for the discovery of novel signalling proteins and small molecule inhibitors (Uvebrant *et al.* 2007) such as the ADAM15-binding sorting nexin family member SNX30 (Karkkainen *et al.* 2006).

In this study, the recombinant ADAM15 disintegrin domain (RADD) was expressed in *E. coli*, and 4 specific binding peptides of RADD were obtained by positive panning and subtractive selection using a phage display 12-mer (Ph.D.-12) library. One of the binding peptides was further shown to inhibit RADD–integrin $\alpha_v\beta_3$ interaction. To the best of our knowledge, this is the first report on the disruption of ADAM15–integrin interactions by short peptides. The identified peptides may have some potential implications for elucidation of an ADAM15 anti-tumour mechanism.

## 2. Materials and methods

### 2.1 *Materials*

The Ph.D.-12 Phage Display Peptide Library Kit (#E8110SC; New England Biolabs, Beverly, USA) contains random 12-mer peptides with a complexity of $2.7 \times 10^9$ different presented peptides and has a concentration of $1.5 \times 10^{13}$ phages/ml (pfu/ml). The horseradish peroxidase (HRP)-conjugated anti-M13 antibody was from Pharmacia Biotech (Sweden). Streptavidin MagneSphere® Paramagnetic Particles and a magnetic stand were obtained from Promega Corporation (Madison, Wisconsin, USA). ProtOn Biotin Labeling Kit (Vector) was purchased from Jingmei BioTech Co. Ltd. (Shanghai, China). All other chemicals were of analytical grade and are commercially available.

### 2.2 *Strains, cells and culture conditions*

*E. coli* Rosetta (DE3) (Invitrogen) was used to produce the RADD protein, and the microorganism was cultivated on Luria–Bertani (Fleming *et al.* 2005) medium with ampicillin (100 $\mu$g/ml) and chloramphenicol (50 $\mu$g/ml). A375M human melanoma cells obtained from the American Type Culture Collection (ATCC) were maintained in Dulbecco modified Eagle medium (DMEM, Life Technologies, USA) supplemented with 10% foetal calf serum (FCS). Human microvascular endothelial cells (HMEC-1) kindly provided by Dr He Lu (Inserm U553, Hospital Saint-Louis, Paris, France) were grown as monolayers in MCDB 131 medium (Sigma) supplemented with 2 mM L-glutamine, 100 IU/ml penicillin, 100 $\mu$g/ml streptomycin, 10% FCS, 10 ng/ml epidermal growth factor (EGF), and 1 $\mu$g/ml hydrocortisone (Sigma).

### 2.3 *Expression and purification of the RADD protein*

The cDNA that encodes the entire disintegrin domain of ADAM15 was inserted between the *Bam*HI and *Not*I sites of the pGEX-4T-1 vector, which was then transformed into *E. coli* Rosetta (DE3). The glutathione *S*-transferase (GST)–RADD fusion protein was expressed by inducing the bacteria with 0.1 mM isopropyl-1-thio-$\beta$-D-galactopyranoside (IPTG) for 6 h. GST–RADD was extracted by sonicating the bacteria and purified by Glutathione Sepharose 4B affinity chromatography (Pharmacia, Sweden). RADD was released from GST–RADD by thrombin cleavage (10 U thrombin/mg fusion protein) at 4°C for 8 h. The resultant protein mixture was further separated by Sephadex G-75 gel filtration chromatography (500 g, Pharmacia). Purified RADD was concentrated, and the protein concentration was determined by the BCA Protein Assay Kit (Pierce Chemical). The molecular weight of RADD was determined by a matrix-assisted laser desorption ionisation time of flight (MALDI-TOF) mass spectrometer (4700 Proteomics Analyzer, Applied Biosystems, Foster City, CA, USA). RADD specificity was determined by western blot analysis using the anti-human ADAM15 ectodomain antibody (R&D Systems Co. Ltd.), according to a standard procedure.

### 2.4 *Cell adhesion assay*

Matrigel was coated on 24-well plates (5 $\mu$g/well) and left overnight at 4ºC; the plates were washed 3 times with phosphate buffered saline (PBS), blocked with 1% bovine serum albumin (BSA) in PBS for 2 h, and rinsed with PBS. A375M cells expressing $\alpha_v\beta_3$ were resuspended in DMEM medium with or without RADD at a concentration of $1 \times 10^4$ cells/well and incubated for 2 h. After removing unattached cells by washing, the attached cells were detached with trypsin-EDTA and then counted. HMEC-1 cells were used as controls. Saxatilin, a typical disintegrin containing an RGD motif, is known to interact with $\alpha_v\beta_3$; it was used as the positive control, while BSA served as the negative control.

### 2.5 *Immobilisation of biotinylated RADD protein*

The RADD protein was biotinylated using the ProtOn Biotin Labeling Kit, according to the manufacturer's instructions. For immobilisation of the biotinylated RADD (b-RADD)

protein, 1 mg streptavidin-coated magnetic beads were washed and resuspended in 100 $\mu$l PBS (pH 7.5) in a 1.5 ml Eppendorf tube. Subsequently, 500 $\mu$l PBS-diluted b-RADD (100 $\mu$g/ml) was added. The resulting preparation was gently mixed on an end-to-end laboratory shaker at 4°C for 2 h. After incubation, the particles were separated from the mixture using the magnetic stand. The supernatant was removed, and the magnetic particles bound to b-RADD were washed 3 times with 1 ml Tris-buffered saline Tween-20 (TBST).

### 2.6 *Biopanning*

One millilitre of $1.5 \times 10^{11}$ pfu phages were added to the b-RADD-coated magnetic particles after blocking with 1 ml 5% skimmed milk for 1 h at 25°C. The mixture was incubated for 2 h at 25°C with gentle shaking. Next, unbound phages were removed by approximately 6 washes with TBST buffer. The bound phage particles were eluted with 0.2 M glycine-HCl (pH 2.2), neutralised with 1 M Tris-HCl (pH 9.1), and used for the titre assay and to infect *E. coli* ER2738 for amplification. The phage titre was evaluated by the blue plaque-forming assay on an agar plate containing IPTG and X-gal. To amplify selected phage clones, the phages were mixed with 20 ml *E. coli* ER2738 culture and incubated at 37°C with vigorous shaking for 4.5 h. After the first round of panning, a subtraction round was performed using the streptavidin magnetic particles without b-RADD in the same manner as before, in order to separate streptavidin-binding phages. The residual phages were amplified and titred for the next round of panning. After 4 rounds of positive panning and 2 rounds of subtractive screening, 40 single plaques were selected for DNA sequencing.

### 2.7 *Phage enzyme-linked immunosorbent assay*

Enzyme-linked immunosorbent assay (ELISA) was performed to compare the binding activity of different peptide sequences. Streptavidin was coated onto 96-well plates one day before use. b-RADD dissolved in $NaHCO_3$ (0.1 M, pH 8.6) was added to the wells (50 $\mu$g/ml, 100 $\mu$l/well) followed by incubation at 25°C for 2 h. After washing with TBST, phage particles diluted with 0.3% BSA-blocking buffer were added to the wells ($10^{10}$ pfu/well) and incubated for 2 h at 25°C. After washing, the HRP-anti-M13 antibody (diluted 1:5000 in BSA-blocking buffer) was added, and the plate was incubated for 2 h. Subsequently, the plates were washed 5 times with TBST buffer, followed by the addition of the ABTS substrate and $H_2O_2$ and further incubated for 20 min. The absorbance at 405 nm was measured with a microplate reader (MultiskanMK3, Thermo Labsystems). Wells coated with streptavidin or streptavidin–biotin without the RADD protein were used as control.

### 2.8 *Solid-phase binding assays*

Solid-phase binding assays were performed using a modification of the method of Nath *et al.* (1999). Purified human integrin $\alpha_v\beta_3$ was diluted to 5 $\mu$g/ml with Hanks balanced salt solution (HBSS) and added to a 96-well ELISA plate (Life Technologies, USA). The plates were incubated overnight at 4°C and blocked with 200 $\mu$l 5% BSA in HBSS for 2 h at room temperature. The wells were then washed 3 times in HBSS containing 1 mM $MnCl_2$ and 1 mg/ml BSA (Buffer A). Fifty-micro litre samples of RADD or saxatilin (5 $\mu$g/ml) were added with or without synthetic peptide A, and the plate was incubated for 3 h at 30°C. The wells were washed 3 times in Buffer A, and bound RADD or saxatilin was detected by adding the anti-human ADAM15 ectodomain antibody (1:1000) in Buffer A for 30 min at room temperature. The wells were then washed 4 times in Buffer A, and the colour was developed using o-phenylenediamine dihydrochloride (OPD) as the substrate (Sigma, USA). Absorbance at 450 nm was measured using a multi-well microtitre plate reader.

## 3. Results

### 3.1 *RADD preparation*

The soluble GST–RADD protein expressed in recombinant *E. coli* Rosetta (DE3) was obtained by Glutathione Sepharose affinity chromatography and cleaved by thrombin. The reaction mixture was then passed through a Sephadex G-75 gel filtration chromatography with PBS (pH 7.4) at 2.5 ml/min. The purified RADD was collected, and migrated as a single band on a tricine gel. Subsequently, a series of experiments was performed to confirm the purified RADD protein: (i) The purity of RADD analysed by high-performance liquid chromatography (HPLC) with SephadexG-75 was more than 95%. (ii) Purified RADD could be immunoreactive with anti-human ADAM15 antibody (figure 1a). (iii) The molecular weight of the RADD protein was determined to be approximately 11107.47 by MALDI-MS analysis, and the protein appeared as a single major peak (figure 1b). The amino acid analysis showed correct in-frame protein translation, and the amino acid residues were identical to those of the native protein (MAAFCGNMFVEPGEQCD CGFLDDCVDPCCDSLTCQLRPGAQCASDGPCCQNC QLRPSGWQCRPTRGDCDLPEFCPGDSSQCPPDVSLG DGE).

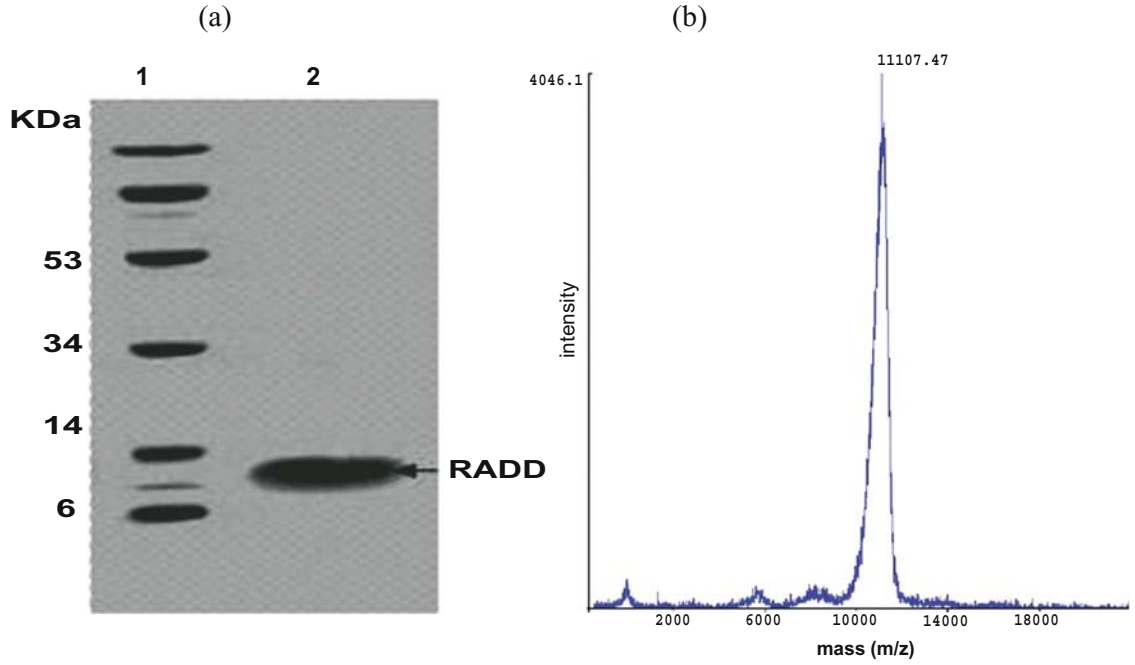


**Figure 1.** (**a**) Western blotting of RADD with the anti-human ADAM15 ectodomain antibody (1:1000). 1, Marker; 2, RADD. (**b**) Mass spectrometry of RADD. Based on the average mass of the major peak, the RADD protein has a molecular weight of 11107.47. The mass per charge is indicated as m/z on the X-axis. Approximately 50 scans were averaged for the analysis.

### 3.2 *Inhibition of melanoma cell adhesion*

Cell adhesion assay was employed to determine the interaction of purified RADD with $\alpha_v\beta_3$. As shown in figure 2, the adhesion of A375M cells expressing $\alpha_v\beta_3$ to matrigel-coated wells was significantly blocked by RADD and saxatilin but not by BSA; further, the inhibitory effect was lower in the case of HMEC-1 cells. Upon incubation with 0.5, 1.0 and 2.0 $\mu$M RADD, the number of adherent A375M cells decreased by 30%, 52% and 80%, respectively, while that of adherent HMEC-1 cells decreased by 5%, 14% and 25%, respectively. These results indicated that RADD strongly affected the integrin $\alpha_v\beta_3$-expressing cells in comparison with the normal cells. Integrin $\alpha_v\beta_3$ played an important role in RADD inhibition of melanoma cell adhesion, and RADD might act as a binding partner of $\alpha_v\beta_3$ to decrease melanoma cell adhesion.

### 3.3 *Enrichment-binding phages of the RADD protein*

The phage clones that were bound to the RADD protein were isolated by 4 rounds of positive panning and 2 rounds of subtractive screening. In each round, the binding phages were rescued and amplified in *E. coli* ER2738 for the next panning round, whereas the unbound phages were removed by washing with TBST. Both phage titre and recovery efficiency were significantly increased after each panning round**.** As shown in figure 3, the number of phages recovered from the RADD protein increased 193-fold (from $1.2 \times 10^2$ pfu to $2.32 \times 10^4$ pfu) compared with that of recovered phages in the first round. The input–output ratio of phages after each round of panning was used to determine the phage recovery efficiency, which increased from $1.87 \times 10^{-7}$ to $1.06 \times 10^{-5}$ (table 1). Approximately 40 phage clones were randomly selected and their DNA sequenced using the primers of $M_{13}$ U**:** 5' GTT CCT TTC TAT TCT CAC TC 3' and $M_{13}$ L**:** 5' TCG TCA CCA GTA CAA ACT AC 3'. The DNA sequences were then translated into amino acid sequencing. Four non-identical peptides, A to D, were obtained (table 2).

### 3.4 *Confirmation of binding by RADD-coated ELISA*

To further confirm the binding ability of the selected phages to the RADD protein, the bacteriophage M13 clones

containing different peptides were randomly chosen (table 1) for ELISA. As shown in figure 4, the absorbance at 405 nm ($A_{405}$) of the wells coated with streptavidin–RADD was much higher than that of the wells in the other two cases, namely, the wells coated with streptavidin and those coated with streptavidin–biotin. This indicated that the binding of the 4 selected phage monoclones to RADD was specific. The clones containing the TPMNHHSQHAER (peptide D) peptide sequence appeared to bind more tightly to the proteins than the other peptide-containing clones. Multiple sequence alignment analyses revealed that peptides A, B and C shared some homology as all of these contained the short motif LIIPP/LPI/IL/P (table 1). Peptide D differs from peptides A, B and C since it lacks a short motif; however, among these 4 peptides, peptide D exhibited the strongest binding to the RADD protein.

### 3.5 *Proteins homologous to the RADD-binding peptide*

Using the BLAST software and a protein database, the following results were obtained: (i) Peptide A exhibits 87% identity with the extracellular segment of integrin $\alpha_v\beta_3$ **(pdb|1L5G|B).** (ii) Peptide B was identical (100%) to the complete extracellular domain of the $\beta$-common receptor of interleukin (IL)-3, IL-5 and granulocyte–macrophage colony-stimulating factor (GM-CSF) **(pdb|2GYS|A)**. (iii) Peptide C was 90% homologous with the tyrosine kinase domain in the hepatocyte growth factor receptor C-Met **(pdb|1R1W|A)**. (iv) Peptide D shared 75% identity with chain A of p38alpha MAPK **(pdb|2BAJ|A).**

### 3.6 *Peptide A inhibition of RADD binding to integrin $\alpha_v\beta_3$*

Since peptide A has 87% identity with the extracellular segment of integrin $\alpha_v\beta_3$ and ADAM15 could interact with integrin $\alpha_v\beta_3$, we tried to identify the effect of peptide A on the interaction between RADD and $\alpha_v\beta_3$. A solid-phase adhesion assay was used to examine the binding of RADD to $\alpha_v\beta_3$ in the presence of synthetic peptide A. As illustrated in figure 5, RADD and saxatilin could specifically bind to $\alpha_v\beta_3$, and anti-$\alpha_v\beta_3$ mAb could significantly block this binding. In comparison with the anti-$\alpha_v\beta_3$ mAb and the negative control, i.e. synthetic peptide D, peptide A unequivocally inhibited the interaction of RADD or saxatilin with $\alpha_v\beta_3$. These results demonstrated that peptide A could disrupt the high affinity RADD–integrin $\alpha_v\beta_3$ interactions. As the target peptide of RADD, peptide A might provide some information on RADD-binding sites on $\alpha_v\beta_3$.

## 4. Discussion

The ADAM15 disintegrin domain containing a unique RGD motif is potentially useful for anticancer treatment. Recently, several studies have focused on the structure–function relationship of this domain (Stone *et al.* 1999; Lu *et al.* 2006; Jeon *et al.* 2007). In this study, RADD was successfully expressed in and purified from recombinant *E. coli* Rosetta (DE3) (figure 1), and its inhibitory effect on the adhesion of melanoma cells expressing integrin $\alpha_v\beta_3$ was also examined *in vitro* (figure 2). A phage display protocol was employed to screen the target peptide of RADD, and 4 specific binding peptides (A to D) were obtained. Based on sequence alignment in the protein database, peptide A was found to have 87% identity with the extracellular segment of integrin $\alpha_v\beta_3$. Synthetic peptide A was demonstrated to inhibit RADD–integrin $\alpha_v\beta_3$ interactions.

The RADD protein contains 15 cysteine residues with multiple disulphide bonds. The cysteine residues may exist freely or form a disulphide bond with the cysteine in the cysteine-enriched domain. Evidence suggests that the extra cysteine residues are functional when the disintegrin-like domain behaves as an adhesion molecule (Wolfsberg *et al.* 1995; Stone *et al.* 1999 ). It is generally suggested that only the RADD protein produced in eukaryotic expression systems is structurally and functionally active. However, in our study, a highly purified and bioactive RADD was obtained by expression in the *E. coli* Rosetta (DE3) strain, and the

**Table 1.** Output assay for each plaque selection round

| Rounds | Concentration of Tween20 (v/v) | Input phages | Titre of recoverd phage (pfu) | Output phages | Recovery ratio (output/input) |
|---|---|---|---|---|---|
| 1 | 0.1 | $1.5\times10^{11}$ | $1.2\times10^{2}$ | $2.8\times10^{4}$ | $1.9\times10^{-7}$ |
| 2 | 0.3 | $1.5\times10^{11}$ | $3.4\times10^{3}$ | $7.6\times10^{5}$ | $5.1\times10^{-6}$ |
| 3 | 0.5 | $1.5\times10^{11}$ | $1.1\times10^{4}$ | $1.2\times10^{5}$ | $0.8\times10^{-5}$ |
| 4 | 0.5 | $1.5\times10^{11}$ | $2{,}3\times10^{4}$ | $1.6\times10^{6}$ | $1.1\times10^{-5}$ |

**Table 2.** Amino acid sequences of target peptides

| Peptide | Sequence (N C) | Frequency |
|---|---|---|
| A | AH***LPI***VRAS***LP***S | 12 |
| B | GN***IIP***DR***P***MH***P***T | 5 |
| C | F***P***SSL***IIPPLP***N | 7 |
| D | T***P***MNHHSQHAER | 16 |

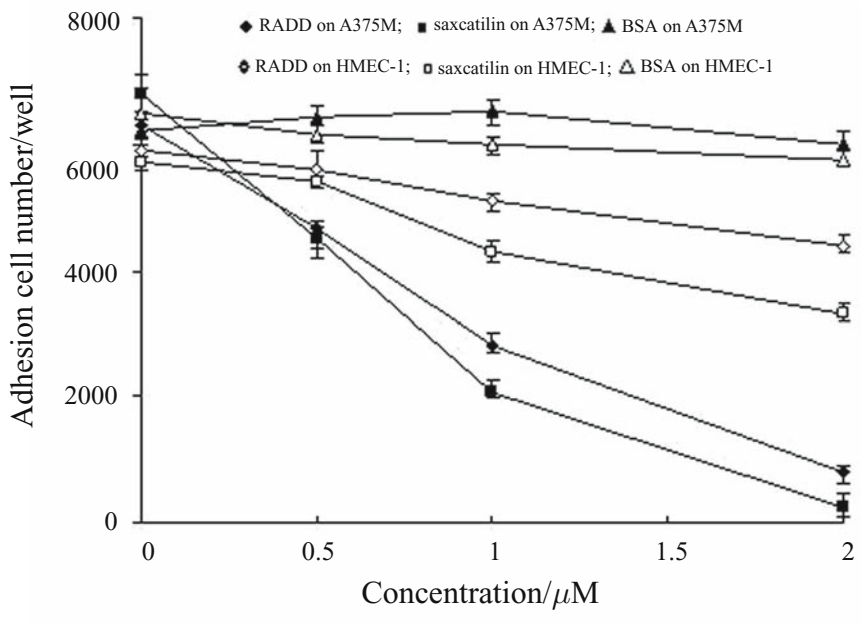


**Figure 2.** Inhibition of melanoma cell adhesion by RADD. HMEC-1 cells were used as the control for normal cells. Saxatilin and bovine serum albumin (BSA) were the positive and negative controls, respectively. Cells were added to each well of matrigel-coated 24-well plates, and treated with various doses of RADD (0.5, 1.0 and 2.0 $\mu$M) for 2 h. Unattached cells were removed by washing with PBS, and adherent cells were detached by treating with trypsin–EDTA for 10 min. The number of adherent cells was counted. Results are expressed as the mean of the number of adherent cells ± SD ($N$ 3). Significance was determined using the Student $t$-test ($^{*}P$<0.01).

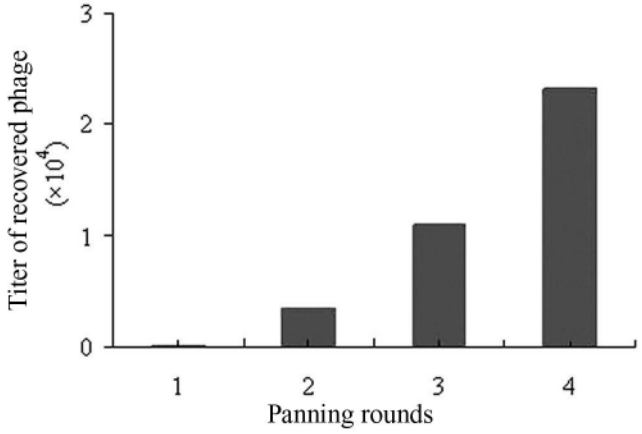


**Figure 3.** Specific enrichment of RADD-binding phages. The titres of the recovered phages from each round were evaluated by the blue plaque-forming assay on an agar plate containing IPTG and X-gal. On the X-axis, the numbers 1 to 4 refer to panning rounds 1 to 4.

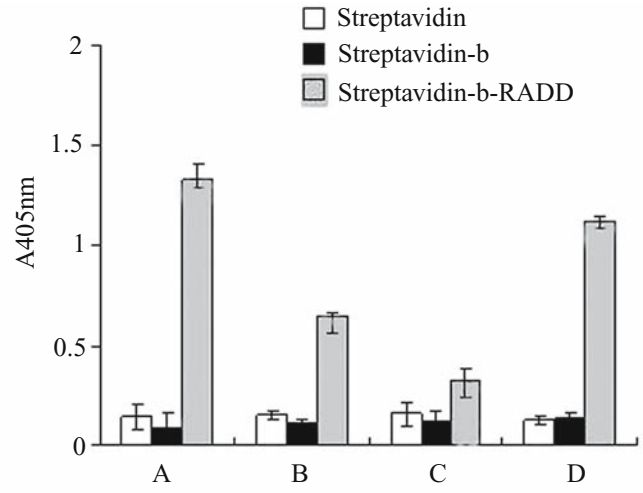


**Figure 4.** Evaluation of RADD binding to selected phages encoding different peptides. Following ELISA, each peptide (A to D) was added to wells coated with either streptavidin–biotin–RADD or with streptavidin or streptavidin–biotin as the control. Binding was detected using the anti-M13 antibody and expressed as the absorbance (405 nm). Results are expressed as the mean ± SD of triplicate experiments. b, biotin

expressed protein had strong inhibitory effects on melanoma cell adhesion *in vitro*. Recombinant ADAM15 disintegrin expressed in *E. coli* exhibited similar physiological behaviour in $\alpha_v\beta_3$-mediated cell–cell interactions, and ASMC adhesion and migration (Zhang *et al.* 1998; Lu *et al.* 2007).

In this study, phage display was performed on the surface of paramagnetic particles, and the results showed that it was effective in studying peptide binding (Steingroewer *et al.* 2007). Using the method of selection and subtraction, false-positives that bind to streptavidin were excluded, and

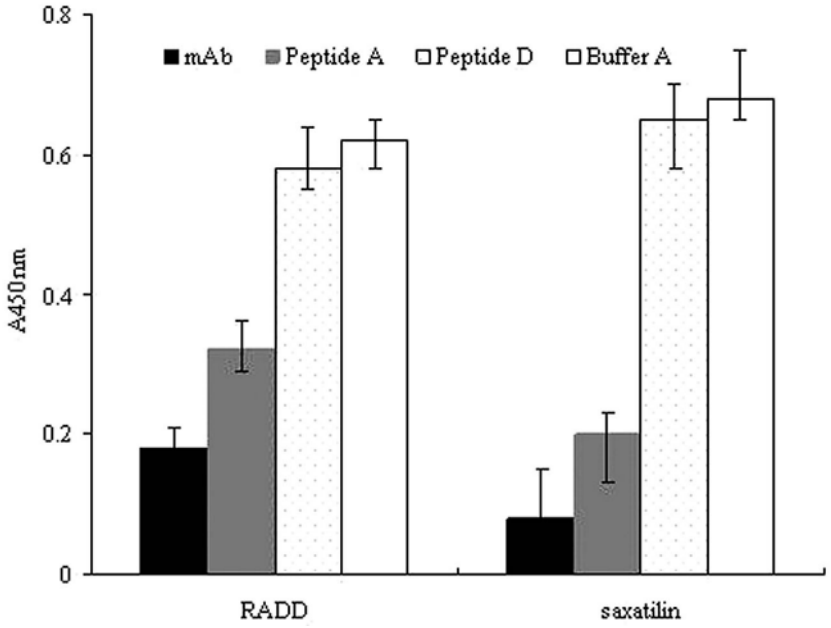


**Figure 5.** Inhibition of RADD binding to integrin $\alpha_v\beta_3$. RADD and the control protein saxatilin were added to wells coated with $\alpha_v\beta_3$ in the presence of peptide A and incubated for 3 h at 30°C. Anti-$\alpha_v\beta_3$mAb and synthetic peptide D were the positive and negative controls, respectively. The anti-human ADAM15 ectodomain antibody was used to detect the bound RADD proteins. The results are expressed as the mean ($A_{450\ nm}$) ± SD of triplicate experiments.

specific peptides that bind to RADD were obtained (table 1). The results from ELISA (figure 4) also confirmed that the 4 peptides specifically bind to RADD. The obtained target peptides were matched in the protein database, and it was interesting to note that peptide A has 87% identity with the extracellular segment of integrin $\alpha_v\beta_3$.

Many studies have reported that integrins such as $\alpha_v\beta_3$, $\alpha_9\beta_1$, and $\alpha_5\beta_1$ are binding partners of ADAM15 (Zhang *et al.* 1998; Trochon-Joseph *et al.* 2004; Beck *et al.* 2005). In the case of ADAM15, it has been proven that the RGD (Arg-Gly-Asp) motif is the binding site for interaction with integrin. The crystal structure of the extracellular segment of integrin $\alpha_v\beta_3$ complexed with a cyclic peptide presenting the RGD sequence has been reported (Xiong *et al.* 2002). However, reports on the actual binding sequence of ADAM15 on these integrins are rare. The results of this study showed that synthetic peptide A could inhibit the interaction of RADD with $\alpha_v\beta_3$. This demonstrated the potential of short peptides in disrupting high-affinity ADAM–integrin interactions. It has been reported that the BAML peptide, which was discovered by phage display, can act as a specific antagonist of cytokine–BLyS receptor interactions, and the binding sites on the laminin receptors are components of antibiotics (Fleming *et al.* 2005; Kobayashi and Yoshida 2007). On the other hand, A375M melanoma cells are known to express high levels of $\alpha_v\beta_3$; therefore, it is conceivable that RADD targeted $\alpha_v\beta_3$ in A375M cells to inhibit its adhesion. However, the relationship of peptide A with the RADD-binding site on $\alpha_v\beta_3$ requires further study.

In conclusion, four specific target peptides of RADD were selected by phage display, and peptide A was found to inhibit the interaction between RADD and $\alpha_v\beta_3$. To the best of our knowledge, a short peptide has been used for disrupting high-affinity ADAM–integrin interactions for the first time. Our study also has some potential implications for characterising the anticancer molecular mechanisms of ADAM15, and developing novel and efficient anticancer drugs.

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