

# A novel peptide, PLAIDGIELTY, for the targeting of $\alpha_9\beta_1$ -integrins

Holm Schneider<sup>a,\*</sup>, Richard P. Harbottle<sup>a</sup>, Yasuyuki Yokosaki<sup>b</sup>, Jan Kunde<sup>a</sup>,  
Dean Sheppard<sup>c</sup>, Charles Coutelle<sup>a</sup>

<sup>a</sup>Gene Therapy Research Group, Section of Molecular Genetics, Division of Biomedical Sciences,  
Imperial College School of Medicine at St Mary's Hospital, Norfolk Place, London W2 1PG, UK

<sup>b</sup>Department of Laboratory Medicine, National Hiroshima Hospital, 513 Jike, Saijoh, Higashi-Hiroshima 739-0041, Japan

<sup>c</sup>Lung Biology Center, University of California, San Francisco, CA 94143-0854, USA

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**Abstract** Targeting gene therapy vectors to abundant receptors on airway epithelia may allow a significant enhancement of gene delivery and thereby be of particular importance for the gene therapy of cystic fibrosis.  $\alpha_9\beta_1$ -Integrins are highly expressed throughout the human airway epithelia in vivo, irrespective of any particular clinical status. Aiming to improve the targeting of our non-viral integrin-mediated gene transfer systems to airway epithelia, we searched for a short tenascin C-derived peptide which would bind to these integrins. By utilizing recombinant bacteriophages that display overlapping regions of the third fibronectin type III repeat of tenascin C (TNfn3), we were able to localize its  $\alpha_9\beta_1$ -integrin binding site to the B-C loop of TNfn3. A synthetic Pro-Leu-Ala-Glu-Ile-Asp-Gly-Ile-Glu-Leu-Thr-Tyr peptide (PLAIDGIELTY) was shown to displace  $\alpha_9\beta_1$ -integrin-expressing cells completely from binding to TNfn3. This peptide, therefore, may prove useful both for the examination of the functional importance of  $\alpha_9\beta_1$ -integrins in vivo and the development of gene therapy vectors or drugs targeting these integrins.

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**Key words:**  $\alpha_9\beta_1$ -Integrin; Tenascin; Phage display; Drug targeting; Gene transfer

## 1. Introduction

The recent cystic fibrosis gene therapy trials applying liposomes or adenoviral vectors to airway epithelia have shown that neither the degree nor the duration of corrective effects are yet sufficient to benefit patients significantly (for review see [1]). Current adenoviral gene therapy approaches depend on the cellular expression of  $\alpha_v$ -integrins [2] which are essential for virus entry into the target cells through specific interaction with the arginine-glycine-aspartic acid (RGD) motif of the adenovirus pentone base [3]. Lack of these integrins has been suggested as the reason for the unexpectedly poor performance of adenoviral vectors in recent clinical trials [4–6]. Therefore, the identification of novel ligands targeting abundant receptors on airway epithelial cells may be crucial for further progress in this field of gene therapy, in particular for the development of targeted non-viral vector systems.

\*Corresponding author. Fax: (44) (171) 7063272.  
E-mail: h.schneider@ic.ac.uk

**Abbreviations:** TNfn3, third fibronectin type III repeat of tenascin C; DMEM, Dulbecco's modified Eagle's medium; TBS, Tris-buffered saline; LB, Luria-Bertani; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate; pIII, fd gene III-encoded minor coat protein; BSA, bovine serum albumin; FCS, fetal calf serum

$\alpha_9\beta_1$ -Integrins are highly expressed throughout the human airway epithelia (but not on alveolar epithelium) in vivo, irrespective of any particular clinical status [7]. They have also been found in other tissues where the extracellular matrix glycoprotein tenascin is present, such as the cornea [8] and airway smooth muscle [9], as well as on the surface of cells including hepatocytes which are not surrounded by tenascin [10]. Tenascin C was identified as a specific  $\alpha_9\beta_1$ -integrin ligand and the binding activity could be localized to its third fibronectin type III repeat (TNfn3) [11]. More recently, the N-terminal domain of osteopontin was shown to contain a cryptic adhesive site recognized by  $\alpha_9\beta_1$ -integrins, the sequence of which has not been elucidated yet [12]. It is strongly suspected that other ligands for this receptor also exist [13].

We have previously demonstrated that a cyclic RGD peptide in fusion with oligo-L-lysine is capable of mediating DNA transfer into a variety of cell types in culture [14,15]. We have shown that gene delivery is mediated by integrins, namely the fibronectin and vitronectin receptors. However, as with adenoviral gene delivery systems, our construct would prove incapable of transferring DNA to cells which do not express these receptors. This study, therefore, aimed to find a tenascin C-derived peptide ligand binding to  $\alpha_9\beta_1$ -integrins to improve the targeting of our non-viral vectors to airway epithelia. By combining phage display and competitive cell binding inhibition approaches we identified a novel peptide, PLAIDGIELTY, as promising for the development of gene therapy vectors or drugs targeting  $\alpha_9\beta_1$ -integrins.

## 2. Materials and methods

### 2.1. Cell lines, bacterial strains and reagents

The human colon carcinoma cell line SW480, either mock-transfected or stably expressing  $\alpha_9\beta_1$ -integrins [11], was grown in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum (FCS), penicillin (100 U/ml), streptomycin (100 U/ml) and G418 (1 mg/ml). All tissue culture reagents were purchased from Life Technologies, Paisley, UK.

The bacterial strain *Escherichia coli* TG1 was provided by Dr. Richard Perham (University of Cambridge, UK) and maintained on M9 minimal medium [16].

The pGEX plasmid expressing TNfn3/glutathione S-transferase fusion proteins [17] was obtained from Dr. Kathryn L. Crossin (Scripps Research Institute, La Jolla, CA, USA). Recombinant TNfn3 was produced in *E. coli* and purified as described previously [18].

### 2.2. Oligonucleotides and peptides

Overlapping oligonucleotide pairs TEN1A (5'-TACATGCGGTGCACAGCCCGGGCGCTTGGATGCCCCAGCCAGATC-GAGGTGAAAGATGTACAGACACCACTGCCTTGATCACCTG-3') and TEN1B (5'-TCAGGAGCAGCGGCCCGCCCGGGGTAGGTCAGCTCAATGCCATCGATCTCAGCCAGGGGCTTGA-ACCAGGTGATCAAGGCAGTGGTG-3'), TEN2A (5'-TACATG-

CGGTGCACAGCCCCGGGAGATCGATGGCATTGAGCTGAC-CTACGGCATCAAAGACGTGCCAGGAGACCGTACCACCATCGA-3') and TEN2B (5'-TCAGGAGCAGCGGCCCGCCGGGCTTCAGGTTCCCGATGGAGTCTGGTTCTCGTCTGTGAGATCGATGGTGGTACGGTCTCTCT-3'), TEN3A (5'-TACATGCGGTGCACAGCCCCGGAACCACTCCATCGGGAACTTGAAGCCTGACACTGAGTACGAGGTGTCCCTCATCTCCCGCAG-3') and TEN3B (5'-TCAGGAGCAGCGGCCCGCCGGGTGTTGTGAAGGTCTCTTGGCTGGGTGCTTGACATGTCACTCTGCGGGAGATGAGGGACACC-3'), derived from the cDNA sequence of human tenascin C [19] (adaptor sequences enabling insertion into fd-tet DNA underlined), were purchased from Genosys Biotechnologies, Cambridge, UK. The primer 5'-TGAATTTCTGTATGAGG-3' was used for the sequencing of gene III inserts of fd-tet DNA.

The peptides EIDGIEL, PLAEIDGIELTY, IAEYIPLGLETD and KPLAEI were obtained from Genosys Biotechnologies, the peptide GDIAIEEI was kindly provided by Dr. Nariaki Matsuura (University of Osaka, Japan).

### 2.3. DNA purification, restriction cleavage, ligation and sequencing

The replicating form phage DNA was purified using the QIAprep spin plasmid kit (Qiagen, Crawley, UK), single-stranded DNA by phenol extraction of a phage suspension followed by DNA precipitation with ethanol according to [16]. Restriction cleavage of double-stranded phage DNA, agarose gel electrophoresis and most of the ligations were performed using standard protocols. All restriction endonucleases were products of New England Biolabs, Hitchin, UK. T4 DNA ligase was from Life Technologies. Purification of DNA fragments after separation in an agarose gel was performed with the Qiaex II kit (Qiagen). DNA sequencing was done with the T7 Sequenase 2.0 kit and [<sup>32</sup>P]dATP from Amersham, Bucks, according to the manufacturer's instructions.

### 2.4. Construction of recombinant phages displaying overlapping parts of TNfn3

The bacteriophage fd-tet which confers tetracycline resistance upon transduced cells was a gift from Dr. John McCafferty (Cambridge Antibody Technology, UK).

Oligonucleotide duplexes TEN1, TEN2 and TEN3 were obtained by incubating the oligonucleotide pairs TEN1A/1B, TEN2A/2B and TEN3A/3B, respectively, for 3 min at 90°C, cooling to room temperature within 10 min and filling in using dNTPs and DNA polymerase I large fragment (Boehringer Mannheim, Lewes, UK). The duplexes were cleaved with the restriction endonucleases *Apa*LI and *Nor*I, ligated separately with appropriately cleaved fd-tet DNA backbones and cloned in Subcloning Efficiency DH5 $\alpha$  competent cells (Life Technologies) on LB agar dishes containing 30  $\mu$ g tetracycline/ml LB medium. The DNA sequence and correct insertion of each oligonucleotide duplex were confirmed by sequencing of DNA from single colonies. Phages were amplified in tetracycline-containing LB broth inoculated with transduced bacteria as described in [20]. After pelleting the bacterial cells by centrifugation and decanting the upper 80% of the clarified supernatant, phages were precipitated from the supernatant according to [16]. The precipitate was resuspended in 1/100 supernatant volume of Tris-buffered saline (50 mM Tris-HCl, pH 7.5, 150 mM NaCl). Insoluble material was removed by a further centrifugation at 12 000  $\times$  g. The purified phage suspension was then filter-sterilized and stored at 4°C.

The expected fusion proteins were visualized on a SDS polyacrylamide gel and sufficient pIII function of all phage constructs was demonstrated by transduction of *E. coli* TG1 cells.

### 2.5. Construction of a positive control phage

A positive control phage, encoding the whole TNfn3, was constructed using the oligonucleotide duplexes TEN1, TEN2 and TEN3. TEN1 and TEN2 were joined at the common unique *A*hI site and ligated with a fd-tet DNA backbone at *Apa*LI and *Bam*HI restriction sites in a three-fragment assembly ligation reaction. 1  $\mu$ g DNA in a total volume of 10  $\mu$ l was used in this reaction with a molar ratio of phage DNA to TEN1 and TEN2 of 1:5:5. The ligation procedure comprising two temperature steps was performed as described previously [21] and the resulting phage genome was cloned in *E. coli*. The joined duplexes TEN1 and TEN2 were then excised from replicating form phage DNA with the endonucleases *Apa*LI and

*Sca*LI, isolated from a 2.5% agarose gel and ligated with *Sca*LI/*Bam*HI-cleaved TEN3 and *Apa*LI/*Bam*HI-cleaved fd-tet DNA in another three-fragment assembly ligation reaction followed by transformation of Maximum Efficiency DH5 $\alpha$  competent cells. The presence of the entire TNfn3-DNA in the correct reading frame was established by DNA sequencing. Phages containing the desired insert, named fdTENC, were amplified and purified as described above, and the retained pIII function was confirmed by transduction of *E. coli* TG1 cells.

### 2.6. Phage quantification

The number of infectious fd phages was determined by addition of 10  $\mu$ l samples of 10-fold serial dilutions of the phage suspension in LB to 1 ml of LB broth containing *E. coli* TG1 in mid-logarithmic phase of growth and incubation for 30 min at 37°C under gentle shaking. 100  $\mu$ l of each sample were spread on LB-tetracycline plates, and tetracycline-resistant colonies were counted after incubation for 20 h at 37°C.

### 2.7. Screening of recombinant phages for binding to $\alpha_9\beta_1$ -integrin

The phages fdTENC, fdTEN1, fdTEN2, fdTEN3 and fd-tet were tested separately on both mock-transfected and  $\alpha_9\beta_1$ -integrin-expressing SW480 cells. The cells were harvested from 250 ml tissue culture flasks (Corning) when subconfluent by adding 2 mM EDTA in Dulbecco's phosphate-buffered saline (PBS) and incubating for 20 min at 37°C. Cells were washed with DMEM containing 10% FCS, and the final cell pellet was resuspended in DMEM containing FCS, penicillin/streptomycin and G418. 4  $\times$  10<sup>5</sup> cells/well were transferred to tissue culture-treated 6 well plates and allowed to attach to the wells overnight at 37°C. Wells were washed with PBS on the following day and then blocked with 1% fraction V bovine serum albumin (BSA) in DMEM for 1 h at 37°C. After discarding the blocking solution and repeated washing with Tris-buffered saline (TBS) containing 0.1% v/v Tween-20, 10<sup>11</sup> cfu of the individual phages each in 1 ml TBS containing 0.1% v/v Tween-20 were added and the plates rocked gently for 1 h at room temperature. Afterwards the phage solutions were poured off and the wells were washed thoroughly with TBS containing 0.2% v/v Tween-20. Bound phages were eluted with 1 ml 0.2 M glycine-HCl, 0.1% BSA (pH 2.2) per well by gentle rocking for 10 min at room temperature. The eluates were transferred to individual tubes and neutralized each with 150  $\mu$ l 1 M Tris-HCl (pH 9.1). The number of eluted phages/well was estimated by separate titring as described above.

### 2.8. Competitive cell binding inhibition assays

Individual wells of non-tissue culture-treated polystyrene 96 well flat bottom microtiter plates (Costar EIA/RIA 3590) were coated by incubation with intact human or chicken tenascin C (20  $\mu$ g/ml), purchased from Life Technologies and the Applied Bioligands Corporation (Winnipeg, Canada), respectively, for 12 h at room temperature, or with recombinant TNfn3 for 1 h at 37°C. Wells were washed three times with PBS and then blocked with 1% BSA in PBS for 1 h at 37°C.  $\alpha_9\beta_1$ -Integrin-expressing SW480 cells were harvested from 250 ml tissue culture flasks (Corning) when subconfluent by adding 2 mM EDTA in PBS and incubating for 20 min at 37°C. Cells were washed with DMEM, and the final cell pellet was resuspended in serum-free DMEM containing 0.05% BSA. Aliquots of the cell suspension were pretreated by mixing with the relevant peptides for 30 min at 4°C before addition to the wells. 5  $\times$  10<sup>4</sup> cells were applied to each well, and the plates were incubated for 1–1.5 h at 37°C in a humidified atmosphere with 5% CO<sub>2</sub>. After washing the wells several times with PBS, attached cells were fixed with pure methanol and stained with 0.1% (w/v) crystal violet [22]. Excess stain in the wells was thoroughly washed away with PBS. After air-drying the cells were solubilized in 1% SDS and quantified by measuring the absorbance at 570 in a microplate spectrophotometer (SLT).

## 3. Results and discussion

The phage display technology has proven useful for the identification of cell surface receptor binding peptides [23–25]. By fusing DNA with the minor coat protein pIII gene of filamentous bacteriophages, phages candidate peptides can be displayed on the surface of the phages, which can be ap-

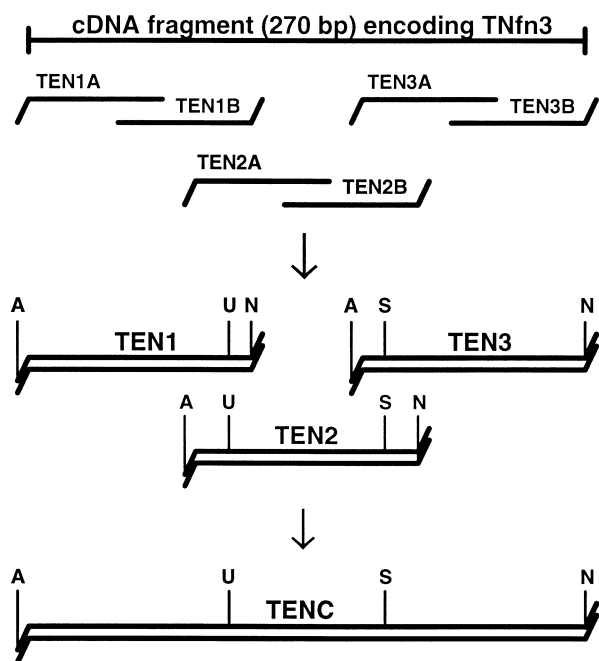


Fig. 1. Strategy for the construction of recombinant phages displaying overlapping parts of the third fibronectin type III repeat of tenascin C. Six oligonucleotides derived from the cDNA encoding human tenascin C were used to generate three phages displaying overlapping peptides of 36 amino acids each and a control phage exposing the entire TNfn3. Oligonucleotide duplexes were obtained by annealing of the oligonucleotide pairs TEN1A/1B, TEN2A/2B and TEN3A/3B and filling-in using DNA polymerase I large fragment. The duplexes were cleaved with the restriction endonucleases *Apa*LI and *Not*I and cloned separately in fd phages, resulting in the phages fdTEN1, fdTEN2 and fdTEN3. Besides, the duplexes TEN1 and TEN2 were joined at the common unique *Alu*I site and sub-cloned in fd phage. The joined fragments were then excised from the vector and ligated with duplex TEN3 at the common unique *Sca*I site, and the product TENC encoding the entire TNfn3 was cloned in the phage. Adaptor sequences enabling insertion into phage DNA are indicated by hooks pointing upwards and downwards. Vertical lines represent restriction sites for *Apa*LI (A), *Not*I (N), *Alu*I (U) and *Sca*I (S).

plied to the target of interest. To display three overlapping peptides of the entire TNfn3 in fusions with the coat protein pIII of the filamentous bacteriophage fd-tet, we constructed the recombinant phages fdTENC, fdTEN1, fdTEN2, fdTEN3 according to the strategy shown in Fig. 1. Expression of the fusion proteins was verified by SDS-PAGE of the phage proteins (data not shown), and sufficient pIII function of all phage constructs was demonstrated by transduction of TG1 cells.

The phages fdTENC, fdTEN1, fdTEN2, fdTEN3 and fd-tet were applied separately at equal titers to  $\alpha_9\beta_1$ -integrin-expressing SW480 cells, and tested in parallel on mock-transfected control cells, which do not express  $\alpha_9\beta_1$ -integrins. Both phages fdTEN1 and fdTEN2, but not fdTEN3, were found to bind specifically to the  $\alpha_9\beta_1$ -integrin-expressing SW480 cells (Fig. 2A), suggesting the location of the  $\alpha_9\beta_1$ -integrin binding site in the overlapping region of fdTEN1 and fdTEN2 (Fig. 2B). These data are consistent with the results of a parallel mutational analysis of acidic amino acid residues in TNfn3 by Yokosaki and co-workers [18], which identified the same region as critical for the binding of tenascin C to  $\alpha_9\beta_1$ -integrins.

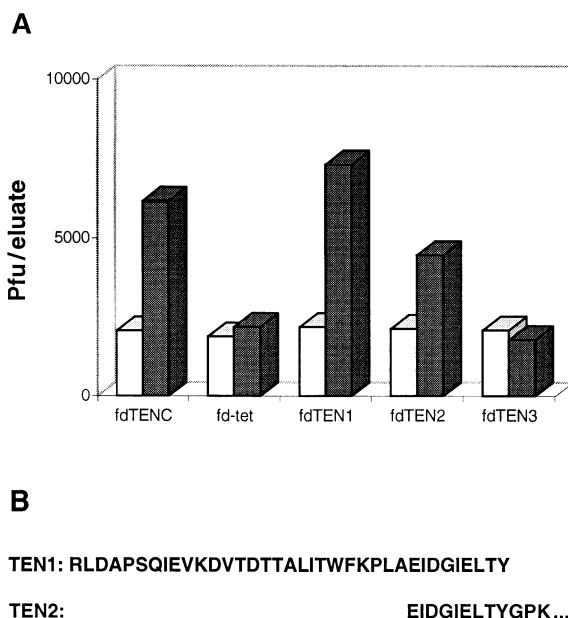


Fig. 2. Adhesion of fd phages displaying overlapping parts of the third fibronectin type III repeat of tenascin C to transfected SW480 cells. A: Samples of the phages fdTEN-C, fdTEN1, fdTEN2, fdTEN3 and fd-tet at identical titers were applied to mock-transfected (white bars) or  $\alpha_9\beta_1$ -integrin-expressing SW480 cells (black bars) on tissue culture plates blocked with BSA, followed by several washing steps to achieve specific recovery of the binding phages in the final eluate. Afterwards the phage titers in the eluates were estimated. The result is the mean of two separate experiments performed in triplicate for each phage. B: Overlap between the amino acid sequences displayed by the two phages binding specifically to  $\alpha_9\beta_1$ -integrin-expressing cells.

However, since in several independent experiments phage fdTEN1 adhered better to the  $\alpha_9\beta_1$ -integrin-expressing cells than fdTEN2, additional amino acid residues appeared to be responsible for a strengthening of that binding. After sub-

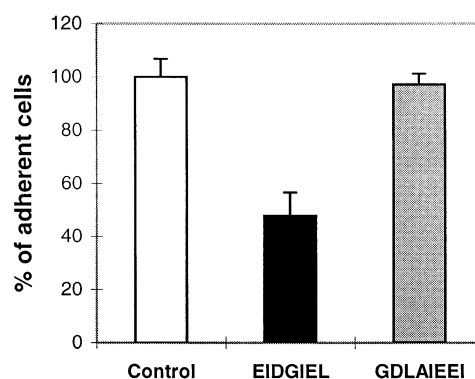


Fig. 3. Competitive inhibition of the binding of  $\alpha_9\beta_1$ -integrin-expressing SW480 cells to human tenascin C by a synthetic EIDGIEL peptide. Microtiter plates were coated with intact human tenascin C (20  $\mu$ g/ml) and blocked with BSA.  $\alpha_9\beta_1$ -Integrin-expressing SW480 cells, either native (white bar) or pretreated with the indicated peptides in equimolar amounts, were applied to the wells, and the plates were incubated for 1.5 h at 37°C, 5% CO<sub>2</sub>. After washing the wells, attached cells were fixed with methanol, stained with crystal violet, solubilized in 1% SDS and quantified by measuring the absorbance at 570 nm. Values are shown as percentage of the untreated control cells. The result is the mean of four separate experiments performed in triplicate, error bars represent the standard deviations.

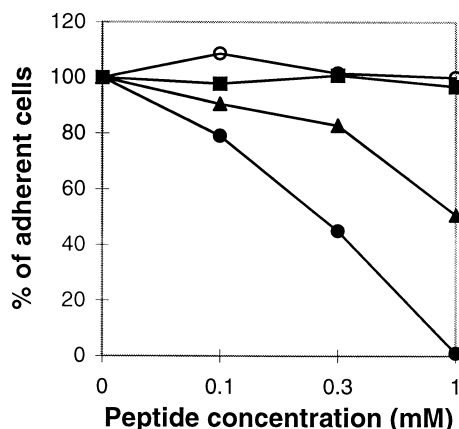


Fig. 4. Effects of different synthetic peptides on the adhesion of  $\alpha_9\beta_1$ -integrin-expressing SW480 cells to recombinant TNfn3.  $\alpha_9\beta_1$ -Integrin-expressing SW480 cells, either native or pretreated with three different concentrations (0.1, 0.3 or 1 mM) of the peptides EIDGIEL (▲), PLAIEDGIELTY (●), IAEYIPLGLETD (○) or KPLAEI (■), were applied to microtiter plates coated with recombinant TNfn3 (3  $\mu\text{g}/\text{ml}$ ). The plates were incubated for 1 h at 37°C, 5%  $\text{CO}_2$ . After washing the wells, attached cells were fixed, stained with crystal violet, solubilized and quantified by measuring the absorbance at 595 nm. Each point is the mean of triplicate wells from a representative experiment.

traction of the background the difference in the binding capabilities of fdTEN1 and fdTEN2 was about 50% on average.

In accordance with the overlap between TEN1 and TEN2 we first synthesized the heptapeptide EIDGIEL and studied its interactions with  $\alpha_9\beta_1$ -integrin-expressing SW480 cells in competition with human tenascin C. At a concentration of 1 mM, this peptide was found to inhibit cell adhesion by 50%, whereas a scrambled control peptide did not influence that binding (Fig. 3). However, its homologous region in chicken tenascin C (a recombinant fragment of which was used for the mutagenesis approach by Yokosaki and co-workers [18]) contains a glutamic acid residue instead of the aspartic acid residue, and the cells used in both studies express human  $\alpha_9\beta_1$ -integrins. Therefore, we confirmed that this amino acid exchange is not critical by performing an analogous competitive cell binding inhibition experiment on plates coated with native chicken tenascin C, and obtained virtually the same results as with the human protein (data not shown). As the two negatively charged amino acids are exchangeable, only their charge, which could provide an alternative coordination group for the integrin to chelate a divalent cation, may play a role in the binding process. This would be consistent with the assumption that interaction with receptor-bound cations is the common mechanism for ligand binding to integrins [26].

However, since the peptide EIDGIEL did not show a binding potency likely to be useful for our purposes and a region displayed only by phage fdTEN1 appeared to be responsible for a strengthening of the EIDGIEL-mediated binding, we extended our investigations to N-terminal amino acids adjacent to EIDGIEL. In tenascin C the sequence Pro-Leu-Ala-Glu-Ile-Asp-Gly (PLAEIDG) is found exposed in a loop between strands B and C connecting the two  $\beta$ -sheets of TNfn3 [27]. To test the hypothesis that the considerable stronger binding of fdTEN1 in comparison with fdTEN2 may be due to the presence of the entire amino acid sequence of this loop, we synthesized the peptide PLAIEDGIELTY representing the

complete overlap between TEN1 and TEN2 extended by the remaining three amino acids of the B-C loop of TNfn3. We also synthesized the peptide KPLAEI to clarify whether the additional amino acid sequence contains  $\alpha_9\beta_1$ -integrin binding activity on its own. The effects of all peptides were directly compared in a competitive cell binding inhibition experiment on plates coated with recombinant TNfn3. Pretreatment of  $\alpha_9\beta_1$ -integrin-expressing cells with PLAIEDGIELTY at a concentration of 1 mM resulted in a complete displacement of the cells from binding to TNfn3, whereas a scrambled control peptide (IAEYIPLGLETD) did not inhibit that binding. Furthermore, there were significant differences between the blocking effects of EIDGIEL and PLAIEDGIELTY at all peptide concentrations tested (Fig. 4). Since the difference in the binding capabilities of phages fdTEN1 and fdTEN2 was reproduced by the peptides PLAIEDGIELTY and EIDGIEL, it is most likely due to the amino acid sequence Pro-Leu-Ala. Pretreatment of the cells with KPLAEI did not influence the adhesion of  $\alpha_9\beta_1$ -integrin-expressing cells to TNfn3. Thus, the N-terminal part of the B-C loop without the aspartate and subsequent amino acid residues is unable to mediate  $\alpha_9\beta_1$ -integrin binding on its own, suggesting that the amino acids proline, leucine and alanine function as part of the recognition site through fulfillment of conformational requirements for the integrin binding. However, we cannot exclude an unexpected additional contribution of the threonine and tyrosine residues which, since they are situated far away from the exposed loop, are unlikely to belong to the cell adhesion site.

The extended peptide found in this study may prove useful both for the examination of the functional importance of  $\alpha_9\beta_1$ -integrins in vivo and for the development of gene therapy vectors or drugs targeting these integrins.

Integrins often have specific extracellular matrix proteins as ligands, but many individual integrins can also interact with cell surface proteins, and most members of the integrin family can recognize more than one ligand [28]. Therefore, we will continue to search for short peptides that bind specifically to  $\alpha_9\beta_1$ -integrins by biopanning against  $\alpha_9\beta_1$ -integrin-expressing SW480 cells using a random peptide phage display library. In accordance with the aim of our study to identify a novel targeting ligand for airway epithelia, we will now investigate the ability of PLAIEDGIEL peptides covalently linked to polycations to mediate specific gene delivery to  $\alpha_9\beta_1$ -integrin-expressing cells.

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## References

- [1] Coutelle, C. (1997) in: Concepts in Gene Therapy (Strauss, M. and Barringer, J.A., Eds.), pp. 315–345, De Gruyter, Berlin.
- [2] Goldman, M.J. and Wilson, J.M. (1995) *J. Virol.* 69, 5951–5958.
- [3] Wickham, T.J., Mathias, P., Cheresch, D.A. and Nemerow, G.R. (1993) *Cell* 73, 309–319.
- [4] Grubb, B.R., Pickles, R.J., Ye, H., Yankaskas, J.R., Vick, R.N., Engelhardt, J.F., Wilson, J.M., Johnson, L.G. and Boucher, R.C. (1994) *Nature* 371, 802–806.

- [5] Zabner, J., Freimuth, P., Puga, A., Fabrega, A. and Welsh, M.J. (1997) *J. Clin. Invest.* 100, 1144–1149.
- [6] Takayama, K., Ueno, H., Pei, X.-H., Nakanishi, Y., Yatsunami, J. and Hara, N. (1998) *Gene Ther.* 5, 361–368.
- [7] Weinacker, A., Ferrando, R., Elliott, M., Hogg, J., Balmes, J. and Sheppard, D. (1995) *Am. J. Respir. Cell Mol. Biol.* 12, 547–557.
- [8] Tervo, T., van Setten, G.B., Lehto, I., Tervo, K., Tarkkanen, A. and Virtanen, I. (1990) *Ophthalmol. Res.* 22, 128–133.
- [9] Soini, Y., Paakko, P., Nuorva, K., Kamel, D., Linnala, A., Virtanen, I. and Lehto, V.P. (1993) *Am. J. Clin. Pathol.* 100, 145–150.
- [10] van Eyken, P., Geerts, A., de Bleser, P., Lazou, J.M., Vrijssen, R., Sciôt, R., Wisse, E. and Desmet, V.J. (1992) *Hepatology* 15, 909–916.
- [11] Yokosaki, Y., Palmer, E.L., Prieto, A.L., Crossin, K.L., Bourdon, M.A., Pytela, R. and Sheppard, D. (1994) *J. Biol. Chem.* 269, 26691–26696.
- [12] Smith, L.L., Cheung, H.-K., Ling, L.E., Chen, J., Sheppard, D., Pytela, R. and Giachelli, C.M. (1996) *J. Biol. Chem.* 271, 28485–28491.
- [13] Sheppard, D. and Yokosaki, Y. (1996) *Chest* 109, 29S–33S.
- [14] Harbottle, R.P., Cooper, R., Hart, S., Ladhoff, A., McKay, T., Knight, A.M., Wagner, E., Miller, A. and Coutelle, C. (1998) *Hum. Gene Ther.* (in press).
- [15] Hart, S., Harbottle, R.P., Cooper, R., Miller, A., Williamson, R. and Coutelle, C. (1995) *Gene Ther.* 2, 552–554.
- [16] Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
- [17] Prieto, A.L., Edelman, G.M. and Crossin, K.L. (1993) *Proc. Natl. Acad. Sci. USA* 90, 10154–10158.
- [18] Yokosaki, Y., Matsuura, N., Higashiyama, S., Murakami, I., Obara, M., Yamakido, M., Shigeto, N., Chen, J. and Sheppard, D. (1998) *J. Biol. Chem.* 273 (in press).
- [19] Nies, D.E., Hemesath, T.J., Kim, J.-H., Gulcher, J.R. and Stefansson, K. (1991) *J. Biol. Chem.* 266, 2818–2823.
- [20] McCafferty, J., Griffiths, A.D., Winter, G. and Chiswell, D.J. (1990) *Nature* 348, 552–554.
- [21] Schneider, H., Tolmachov, O., Kunde, J. and Coutelle, C. (1998) *Biochemica* (in press).
- [22] Brasaemle, D.L. and Attie, A.D. (1988) *BioTechniques* 6, 418–419.
- [23] Barry, M.A., Dower, W.J. and Johnston, S.A. (1996) *Nature Med.* 2, 299–305.
- [24] Koivunen, E., Wang, B. and Ruoslahti, E. (1994) *J. Cell Biol.* 124, 373–380.
- [25] Cwirla, S.E., Peters, E.A., Barrett, R.W. and Dower, W.J. (1990) *Proc. Natl. Acad. Sci. USA* 87, 6378–6382.
- [26] Humphries, M.J. (1990) *J. Cell Sci.* 97, 585–592.
- [27] Leahy, D.J., Hendrickson, W.A., Aukhil, I. and Erickson, H.P. (1992) *Science* 258, 987–991.
- [28] Hynes, R.O. (1992) *Cell* 69, 11–25.