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Identification and validation of a novel cell-recognition site (KNEED) on the 8th type III domain of fibronectin

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Abstract

Interactions between cell-surface integrins and extracellular matrix proteins underlie a versatile recognition system providing cells with anchorage, traction for migration or matrix remodeling, as well as signals for polarity, differentiation and growth. Short peptide sequences of fibronectin (FN), most notably RGD found on a loop in the 10th type III domain, are effective in promoting cell adhesion when immobilized to a biomaterial scaffold. Additional sequences (e.g. PHSRN) have been shown to act synergistically to enhance cell adhesion and other cellular processes. Using bioinformatics, we identified a candidate cell-binding peptide sequence, KNEED, located on the loop region of the 8th domain of FN that from in vitro studies appears to participate in cell attachment and spreading. Computational analysis revealed that KNEED exhibits both high solvent accessibility and sequence conservation values across FN sequences from seven species. We demonstrate the importance of the KNEED sequence using a solution-phase competitive inhibition assay utilizing soluble peptides. Results indicate that the presence of soluble KNEED peptides inhibits the attachment and spreading of 3T3 balb/c fibroblasts on FN-coated surfaces in a concentration-dependent manner. As more sequence and crystallographic data become available, computational approaches may aid in the identification of new targets for applications where biorecognition plays a key role.

Keywords: Fibronectin; Cell adhesion; Cell spreading; Adhesion peptides

1. Introduction

The identification of minimal requirements for cell adhesion are important for fundamental studies of cell–substrate interactions (e.g. cell adhesion, differentiation, migration) as well as applications in tissue engineering and targeted drug delivery. The identification of RGD in fibronectin (FN) as a key sequence involved in cell adhesion by Pierschbacher and Ruoslahti [1] revolutionized the area of biomaterials because it was subsequently shown that the addition of RGD onto surfaces *alone* is sufficient for cell attachment [2]. However, it has been more difficult to selectively induce cell attachment of specific cell types by RGD alone, and it has also been shown that there can be significant differences in cell behavior between surfaces presenting the intact FN molecule and the RGD peptide [3,4]. Moreover, the RGD sequence also appears in a significant number of cell-binding domains of ECM proteins other than FN (e.g. thrombospondin-I, collagen, laminin, entactin, osteopontin, fibrinogen, vitronectin, and von Willebrand factor), so it is possible that peptide regions *in addition to RGD* confer specificity.

The nature of the interactions between anchorage-dependent cells and extracellular matrix proteins such as FN is a major factor controlling cell behavior. In order for a cell to migrate, grow, or differentiate, it must first attach and spread onto its substrate. The extent of cell spreading is directly related to the adhesion between the cell and its substrate. Cell spreading can be modulated by the addition of soluble peptides. Indeed, addition of soluble Arg–Gly–Asp (RGD) can block FN-mediated adhesion resulting in inhibition of cell spreading and adhesion. This well-studied RGD tripeptide of FN and related adhesion proteins is a common sequence and is conserved across many species of adhesive glycoproteins [5,6].

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Of the 31 domains in the FN protein, the type III domains 8–10 (FN III 8–10) have been classified as cell-binding domains [7]. RGD is located on a loop on the 10th domain of FN (FN III-10), and it has been proposed that a highly conserved loop region on the FN III-9 domain which presents the peptide motif PHSRN acts synergistically with RGD in cell adhesion [8–10]. Moreover, increased cell adhesion is observed when the FN fragment contains the 8th domain (FN III-8) [11], but to our knowledge, there have been no reports of specific cell-binding loops like PHSRN and RGD that have been identified on the FN III-8 domain.

2. Experimental procedures

2.1. Sequence alignment

The crystal structure of the 7th, 8th, 9th, and 10th type III domains of human fibronectin (FN III 7–10) [7] was obtained from the Protein Data Bank (http://www.rcsb.org/pdb; PDB code: 1FNF). An exhaustive search of GenBank (http://ncbi.nlm.nih.gov) for the complete set of sequence data for fibronectin FN III 8–10 (cell-binding domains) uncovered sequences for seven species. Multiple sequence alignment was constructed using PSI-BLAST [12].

The search for a candidate cell-binding peptide was limited to loop regions with the rationale that it is extremely difficult to reproduce secondary structure of proteins in synthetic biomaterial systems whereas loop regions can be relatively easily incorporated. Furthermore, since both RGD and PHSRN are located on loop regions, we hypothesized that good candidate peptide fragments in FN III 8 involved in facilitating cell adhesion are located on loops that are highly conserved and solvent accessible. All loop positions were identified by running a standard protein secondary structure prediction program (DSSP) [13] on the known human FN X-ray structure. The output from DSSP was parsed and all non-secondary structure positions were defined as loops. To compute the solvent accessible area for each residue, the difference in amino acid sizes was taken into account by dividing each area by the average area of the corresponding amino acid [14]. The resulting ratio was found to lie between 0 and 1, but can exceed 1 for highly exposed residues. Sequence conservation at every position was calculated by first identifying the most common amino acid at a position among the seven species, according to the multiple sequence alignment. The percentage of species having the most common residue at each position was calculated, and sequence conservation was defined as the average percentage of the tripeptide centered on the current position.

2.2. Cell culture

3T3 balb/c fibroblasts (ATCC) were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% calf serum, 200 mM L-glutamine and penicillin–streptomycin in 5% CO_2 humidified atmosphere.

2.3. Quantitation of cell number

Cell number was determined using the acid phosphatase assay as developed by Connolly et al. [15]. Briefly, cells were washed with phosphate buffered saline (PBS) cells and subsequently incubated with 10 mM *p*-nitrophenylphosphate (Sigma) in 10 mM sodium acetate, 0.1% Triton X-100, pH 5.5 for 2 h at 37°C. The reaction was stopped with the addition of an equal volume of 0.1 N NaOH and the absorbance (410 nm) was recorded on an Opsys MR microplate reader (Dynex Technologies). A calibration curve was generated relating absorbance to a series of known cell numbers determined independently with a hemocytometer.

2.4. Cell adhesion inhibition assay

Ninety-six well tissue culture plates were coated overnight at 4°C with human plasma FN (Gibco, 0.3 μg/cm^2) dissolved in PBS and blocked for 1 h at 37°C with 1% BSA dissolved in PBS. Cells (between passages 10–18) were trypsinized at 90% confluence and reconstituted in incomplete medium (DMEM supplemented with glutamine, penicillin–streptomycin, and 25 mM HEPES) containing 100 μg/ml soybean trypsin inhibitor (Sigma). Cells were seeded in the presence of soluble peptides (GRGDSP, GRGESP (Gibco); KNEED (New England Peptide)) and incubated at 37°C. After 2 h, non-adherent cells were removed by washing, and adherent cells were either quantified using the acid phosphatase assay or fixed with 3% glutaraldehyde for 1 h.

2.5. Cell-spreading assay

Cell spreading was quantified using image analysis to obtain projected cell areas. Digital images of fixed cells were captured using a Zeiss Axiovert S100 inverted microscope (phase contrast) and a digital camera (Princeton Instruments). Cell areas were measured using the Metamorph Imaging System (Universal Imaging Corporation, Downingtown PA). The cell-spreading assay was calibrated using the areas of 100 non-spread cells treated with 1.0 mM GRGDSP. A cell was classified as "spread" if the cell area is one standard deviation greater than the mean area of non-spread cells ($\sim$300 μm^2). The areas of at least 40–50 cells were determined for each condition.

3. Results and discussion

3.1. Sequence alignment and analysis

The goal of this study was to use structural and sequence alignment information to identify conserved, potential synergistic binding sites for FN that may be involved in modulating cell behavior. Keyword and sequence homology searches of GenBank resulted in a few hundred sequences of FN, tenascin, and hexabrachion, proteins known to have FN type III domains. A large fraction of the resulting hits were fragments of the same sequence. Previous research identified the 8th, 9th, and 10th domains (type III) of human FN as the cell-binding region [6,8], and other studies also included the 7th domain as part of this region. However, the 7th domain was excluded from our analysis due to its decreased conservation across the FN sequences. After careful examination of all FN sequences, we identified seven different species whose sequences included type III domains 8, 9 and 10. Fig. 1 shows that the 8th, 9th, and 10th domains were highly conserved across different species, suggesting important functional purpose of this region to the species. Note that two regions that have been previously identified to be involved in cell adhesion, RGD and PHSRN, are highlighted.

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1235
human     AVPPPTDLRF TNIGPDTMRV TWAPPPSIDL TNFLVRYSPV KNEEDVAELS ISPSDNAVVL
ac frog   AVPPPTNLRF TNIGPDNIRV TWSPPTSIEL SSYLVRYSPV KKPDDVTELS LSPSTNMVVL
rat       AVPPPTDLRF TNIGPDTMRV TWAPPPSIEL TNLLVRYSPV KNEEDVAELS ISPSDNAVVL
newt      CCPTATDLRF TNVGPDSMLV TWSAPPSMVL SSFLVRYVPS KNEEDAAELT ISPSDNMVVL
mouse     AVPPPTDLRF TNIGPDTMRV TWAPPPSIEL TNLLVRYSPV KNEEDVAELS ISPSDNAVVL
chicken   AVPPPTDLRF TNVGPDTMRV TWTAPTSIVL SSFLVRYSPV KKEEDVAELT ISPSDNVVVL
bovine    AVPPPTDLRF TNVGPDTMRV TWAPPSSIEL TNLLVRYSPV KNEEDVAELS ISPSDNAVVL

          1295
human     TNLLPGTEYV VSVSSVYEQH ESTPLRGRQK TGLDSPTGID FSDITANSFT VHWIAPRATI
ac frog   SNLLPFTEYL VSVHSVYEER ESSSLNGVAK THLDSPTGIA FSEITPNSFT VHWIAPRGPI
rat       TNLLPGTEYL VSVSSVYEQH ESIPLRGRQK TGLDSPTGFD SSDVTANSFT VHWVAPRAPI
newt      TNLLPGTEYI VSVFAVYEER ESTPLTGVQR TGLDSPTGLD FSDTTSSSFT VYWMAPRATV
mouse     TNLLPGTEYL VSVSSVYEQH ESIPLRGRQK TGLDSPTGFD SSDITANSFT VHWVAPRAPI
chicken   TNLLPGTEYL VRVYSVAEQH ESAPLSGIQK TGLDSPTGLD FSDITANSFT VHWIAPRATI
bovine    TNLLPGTEYL VSVSSVYEQH ESIPLRGRQK TALDSPSGID FSDITANSFT VHWIAPRATI

          1355
human     TGYRIRHHPE HFSGRPREDR -VPHSRNSIT LTNLTPGTEY VVSIVALNGR EESPLLIGQQ
ac frog   TGYRIRYQLE SGAGRPKEER -VPPSRNSIT LTHLIPGSEY LVSIIAINGQ QESLPLAGQQ
rat       TGYIIRHHAE HSAGRPRQDR -VPPSRNSIT LTNLNPGTEY IVTIIAVNGR EESPPLIGQQ
newt      TGYKIQYHPE TGGAGQKEER CVPPSRNSLT LTNLTPGTEY VVSIFAVNGR QESVPLVGQQ
mouse     TGYIIRHHAE HSVGRPRQDR -VPPSRNSIT LTNLNPGTEY VVSIIAVNGR EESPPLIGQQ
chicken   TGYKIRHHPE HGVGRPKEDR -VPPSRNSIT LTNLLPGTEY VVSIIAVNGR EESVPLVGQQ
bovine    TGYRIRHHPE NMGGRPREDR -VPPSRNSIT LTNLNPGTEY VVSIVALNSK EESLPLVGQQ

          1414
human     STVSDVPRDL EVVAATPTSL LISWDAPAVT VRYYRITYGE TGGNSPVQEF TVPGSKSTAT
ac frog   ATVSDVPTDL EVTSSSPNTL TISWEAPAVS VRYYRITYSQ TGGHGPEKEF TVPGTSNTAT
rat       STVSDVPRDL EVIASTPTSL LISWEPPAVS VRYYRITYGE TGGNSPVQEF TVPGSKSTAT
newt      ATVSDTPTNL EVTSSTPTSM SISWDAPPVG VRYYRITYTE TGGETPVQEF TVPGDRSDAP
mouse     ATVSDIPRDL EVIASTPTSL LISWEPPAVS VRYYRITYGE TGGNSPVQEF TVPGSKSTAT
chicken   TTVSDVPRDL EVNPTSPTSL EISWDAPAVT VRYYRITYGE TGGSSPVQEF TVPGTMSRAT
bovine    STVSDVPRDL EVIAATPTSL LISWDAPAVT VRYYRITYGE TGGSSPVQEF TVPGSKSTAT

          1474
human     ISGLKPGVDY TITVYAVT GRGDSPASSKPI SINYRT
ac frog   IRGLNPGVSY TITVYAVT GRGDSPASSKPL TIIHKT
rat       INNIKPGADY TITLYAVT GRGDSPASSKPV SINYQT
newt      IRGLKPGAEY IITVYAVT GRGDSPASSKPV TVTHKT
mouse     INNIKPGADY TITLYAVT GRGDSPASSKPV SINYKT
chicken   ITGLKPGVDY TITVYAVT GRGDSPASSKPV TVTYKT
bovine    ISGLKPGVDY TITVYAVT GRGDSPASSKPV SINYRT
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Fig. 1. The collection of FN sequences of domains 8, 9, and 10 across seven species: *Homo sapiens* (human), *Xenopus laevis* (african clawed frog), *Rattus norvegicus* (rat), *Pleurodeles waltlii* (iberian ribbed newt), *Mus musculus* (mouse), *Gallus gallus* (chicken), and *Bos taurus* (bovine). Regions of highly conserved amino acids are highlighted in gray. RGD is highlighted within the 10th domain. The synergy region PPSRN (PHSRN in *H. sapiens*) is highlighted within the 9th domain. The KNEED region is highlighted within the 8th domain. Residue numbers according to the X-ray structure are indicated. Residues connecting domains are underlined.

The structural alignment of domains 7–10 (not shown) indicates that these domains share the same overall structure, with functional fragments occupying surface loops. It is of note that the loop regions of the alignment most often contain hydrophilic amino acids since they are positioned on the surface of the protein. Thus, we focused on loop regions for possible binding sites of FN to its integrin counterpart. Using the multiple sequence alignment and the known X-ray structure of human FN type III domains 7–10, the solvent accessibility and residue conservation was computed for all loop positions as described in the Experimental Procedures. Only loop regions of the type III domain 8 are shown (Fig. 2); RGD and PHSRN are shown for reference. The RGD region stands out as 100% conserved over all species and the most solvent accessible. The synergy region PHSRN is also highly conserved, but less solvent accessible. Among the six loops in domain 8, loops 1, 5 and 6 are the most conserved, but are also the least solvent accessible. Visual inspection of the FN X-ray structure indicates that loops 3, 5 and 6 are on the same face as the RGD and the PHSRN regions, while loops 1, 2, 4 are on the opposite face. Fig. 3 shows that KNEED, PHSRN, and RGD are positioned at the same face in the crystal structure of FN. With the above information taken collectively, loop 3 shows the most potential for synergistic behavior. The consensus sequence of loop 3 is KNEED (Fig. 1). Moreover, the KNEED loop on the 8th domain of FN has similar high sequence conservation as RGD and PHSRN (Fig. 2).

Further support for functional significance of KNEED is based on observations made previously by

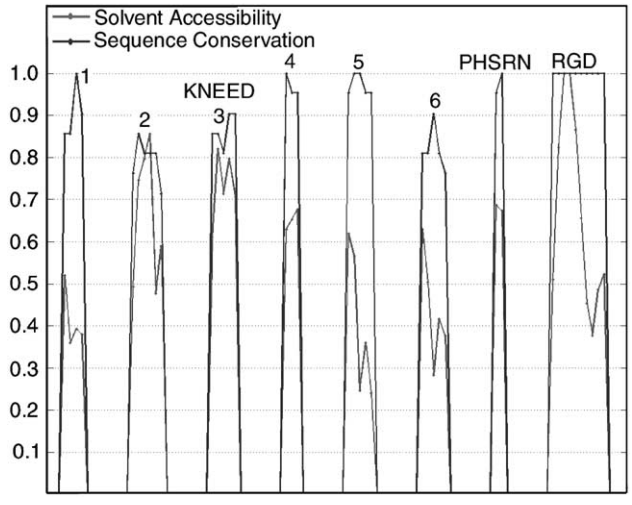

Fig. 2. Computed solvent accessibility and sequence conservation for FN domains 8–10. Only loops are shown, and only RGD is shown for domain 10 and PHSRN for domain 9. The loop number of each of the six loops of domain FN III-8 is indicated in the figure.

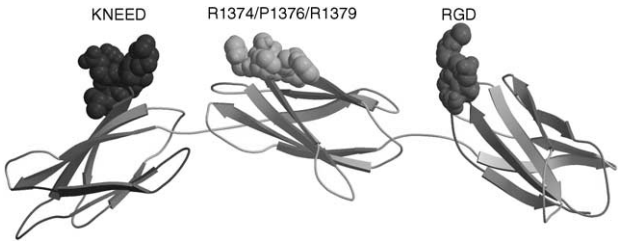

Fig. 3. Ribbon diagram of crystal structure of type III domains 8–10 of FN [17]. RGD is located on the 10th domain and R1374/P1376/R1379 (recently shown by Redick et al. [18] to be the active site in PHSRN) is located on the 9th domain. KNEED is located on the 8th domain. All highlighted sequences are found on loop regions and are depicted here as space-filling models.

researchers that arginine (R) is common to both RGD and PHSRN [8], leading to the conclusion that arginine is partly responsible for cell adhesion. In contrast, the sequence KNEED has only one positively charged amino acid (K; lysine), and is a very negatively charged segment due to the presence of two glutamic acid (E) residues and one aspartic acid (D). It has been shown that electrostatic interactions between various integrin α and β-subunits are important because binding requires the presence of a divalent cation such as Ca^{2+} or Mg^{2+} [3,10]. The cell-binding sequence RGD contains one aspartic acid (D). Thus, we speculate that the negatively charged D residue may interact favorably with the divalent cation. Furthermore, it has been shown that many integrin-binding proteins share the aspartic acid (D) residue (or the related glutamic acid (E) residue), which may provide a missing coordination site when integrin binds to its protein signaling sequence [10]. Based on this argument, the KNEED region of the 8th domain of FN may participate in the binding of cell integrin because of its negative charge.

Other conserved regions of the amino acid sequence are located on the 8th domain of FN but were eliminated from the analysis based on their position relative to neighboring domains and potential for steric hindrance of FN when binding and integrin molecule. A loop region positioned closely to the inner structure of FN or closely to neighboring domains leaves the loop region sequence inaccessible to the integrin molecule and therefore these sheltered regions are most likely not involved in any synergistic activity. Therefore, we hypothesized that KNEED may be of functional importance, and the remainder of this study focuses on the effect of KNEED on cell adhesion.

3.2. Effect of KNEED on cell attachment and spreading

Fig. 4 shows the effect of soluble peptides on cell attachment. Cell attachment decreases sharply with addition of soluble KNEED but appears to plateau at a value of 30% inhibition, compared to GRGDSP

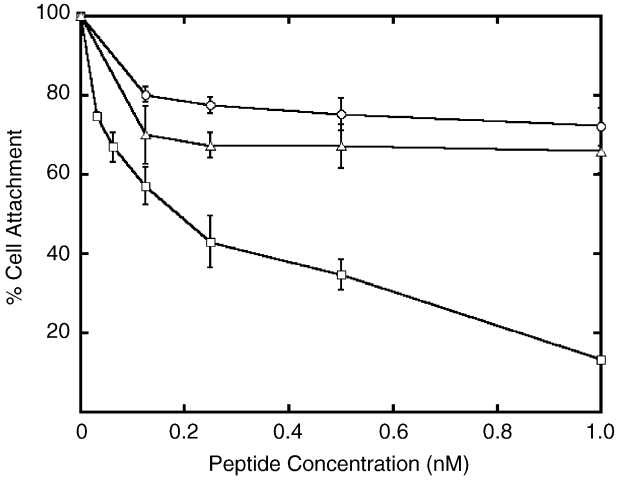

Fig. 4. Inhibition of cell attachment by soluble peptides: 3T3 balb/c cells were seeded onto FN-coated tissue culture plates in the presence of the indicated concentrations of soluble KNEED (○), Ac-KNEED (△), or GRGDSP (□). After 2 h, non-adherent cells were removed by washing, and attached cells were quantified using an acid phosphatase assay.

(RGD) where cell attachment decreases in a concentration-dependent manner to 90% inhibition. As expected, addition of GRGESP does not affect cell attachment (data not shown). The plateau in attachment inhibition suggests that KNEED may have a weaker binding affinity to integrins than RGD. We investigated the effect of removal of extra charge groups on the carboxyl and amino termini of the synthetic KNEED peptide fragments—charges that are not present at these positions in the native FN molecule. However, only a slight increase in cell inhibition from 30% to 35% is observed with the addition of KNEED containing an acetylated N-terminus (Ac-KNEED). For both KNEED and Ac-KNEED, the inhibition of attachment stays at the plateau level for concentrations up to 4 mM (data not shown).

While KNEED does not appear to have a large effect on cell attachment, we observe that KNEED significantly affects cell spreading. The average projected cell areas for the various treatments are listed in Table 1. Note that in general, cells seeded in the presence of soluble KNEED exhibit projected cell areas that are in between that of RGD and RGE. A single-tailed t-test shows that the projected cell area of cells plated with KNEED is significantly different from addition of RGE ($p < 0.005$, Table 1). Numerous studies in the literature have shown that the addition of RGD inhibits cell adhesion, which agrees with the observed decrease in projected cell area of the 3T3 fibroblasts with increasing soluble RGD concentration (Table 1). The addition of RGE leads to minimal changes in projected cell area, although high concentrations (2.5 mM) of peptide appear to lead to some loss in projected cell area (Table 1).

Table 1
Projected cell areas

Peptide concentration (mM)	RGD	KNEED	RGE
0.25	571±38	739±51	998±64
0.5	477±34	791±42	752±51
1.0	379±43	642±67	1112±65
2.5	285±26	385±40	845±102

Values are given as average±SEM.
1.0 mM: RGE vs. KNEED *t*-test, single-tailed ($p<0.005$).

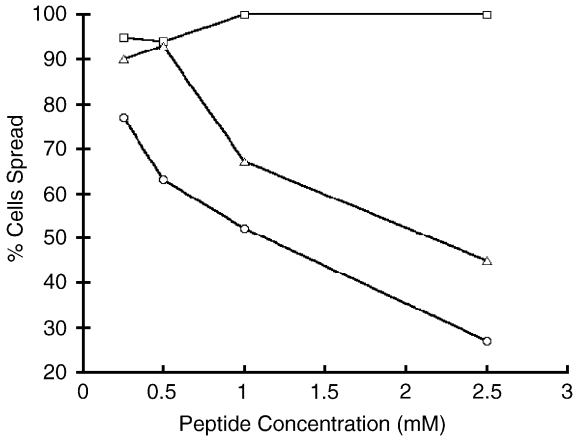

Fig. 5. Inhibition of cell spreading by soluble peptides: 3T3 balb/c cells were seeded in serum-free media onto FN-coated tissue culture plates in the presence of the indicated concentrations of soluble KNEED (△), GRGDSP (○), or GRGESP (□). After 2 h, non-adherent cells were removed by washing, and attached cells were fixed with 3% glutaraldehyde. Phase contrast images of fixed cells were captured using a digital camera and spreading was quantified as described in the Experimental Section.

The effect of KNEED on cell spreading is much more evident when an individual cell is classified as "spread" if its projected cell area is $>350\,\mu m^2$. Fig. 5 shows the effect of soluble KNEED, GRGDSP (RGD), and GRGESP (RGE) on cell spreading. Addition of RGE does not affect cell spreading. However, cultures seeded in the presence of 2.5 mM KNEED and RGD display a 2- and 4-fold reduction in the percentage of spread cells, respectively (Fig. 5). Representative phase contrast images shown in Fig. 6 clearly show that there are significant differences between the morphology of the cells in the presence of KNEED, RGD, or RGE.

Our results suggest that while KNEED may not play a primary role in cell attachment, it may be more important in downstream events that affect cell spreading. Cell attachment and spreading are two distinct processes, and when a cell attaches to a substrate, it does not necessarily mean that it will be a spread cell. However, cell spreading is required for cell viability, i.e. cells which have been prevented from spreading undergo apoptosis [16]. Images of the cells (Fig. 6) are taken after

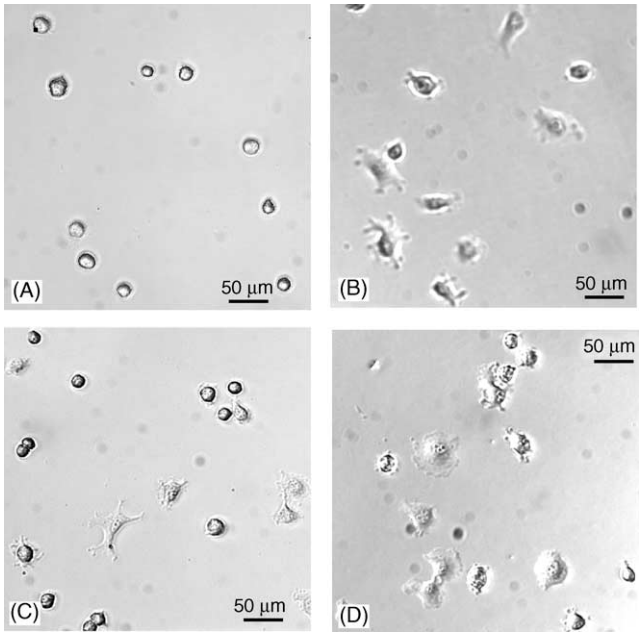

Fig. 6. Phase contrast microscopy: morphology of 3T3 fibroblasts on FN-coated tissue culture-treated polystyrene plates. Images are 2 h after cells were plated in serum-free media after incubation with 1.0 mM soluble (A) GRGDSP, (B) GRGESP, (C) KNEED, or (D) no peptide. Cells were fixed with 3% glutaraldehyde. Scale bar is 50 μm.

sufficient time was allowed for attachment and spreading to occur on FN-coated surfaces. The studies reported here were carried out in serum-free media without growth supplements, and we observe that beyond 2 h, cells begin to exhibit reduced survival (data not shown).

The sequence Lys–Asn–Glu–Glu–Asp (KNEED) is a well conserved loop region on the 8th domain of FN, located 64 Å away from the cell-binding complex RGD. The high sequence conservation and solvent accessibility of the KNEED loop region, similar to that of both the cell-binding region, RGD, and the synergy region, PHSRN, suggests it has functional purpose to the FN molecule. The negatively charged KNEED region suggests it may cooperate with the divalent cation interaction between integrin α and β units, which has been observed to be a characteristic of other integrin-binding motifs (specifically the presence of an aspartic acid) [10]. In addition to sequence conservation, solvent accessibility and the negative charge of the region, the flexibility of the 8th domain suggests that KNEED may be accessible to cell integrin and allow synergistic binding of this sequence along with the RGD complex on the 10th domain.

4. Conclusions

A novel cell-binding site (KNEED) located on a loop of the 8th type III domain of FN was identified using

bioinformatics and protein structural data. Specifically, sequence conservation showed that KNEED is highly conserved in FN across seven different species. Structural data confirmed that KNEED is located on the same face as the known cell-binding regions (RGD and PHSRN).

Decreased cell attachment of 3T3 cells was observed when soluble KNEED was added in the absence of serum. However, inhibition of cell attachment was significantly less (plateauing at 30%) than when soluble RGD was added—indicating that KNEED may have a weaker binding affinity. The addition of soluble acetylated-KNEED resulted in further decrease (35% cell inhibition) in attachment. Although the effect of addition of soluble KNEED on the percent cell attachment is not striking, KNEED significantly affects cell spreading. This is not surprising because cell attachment and spreading are two distinct processes.

The characterization of conserved regions may lead to a better understanding of cell adhesion and cell signaling phenomenon, thus providing a guideline for the design of biologically compatible materials. We show that sequence alignment and analysis can be used to identify peptide fragments that can modulate cell spreading. As more and more sequence and crystallographic data become available, computational approaches combined with biological assays may aid in the identification of new targets for applications where biorecognition plays a key role, most notably targeted drug delivery and tissue engineering.

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References

[1] Pierschbacher MD, Ruoslahti E. Cell attachment activity of fibronectin can be duplicated by small synthetic fragments of the molecule. Nature 1984;309:30–3.
[2] Massia SP, Hubbell JA. An Rgd spacing of 440 nm is sufficient for integrin alpha-v-beta-3-mediated fibroblast spreading and 140 nm for focal contact and stress fiber formation. J Cell Biol 1991;114:1089–100.
[3] Streeter HB, Rees DA. Fibroblast adhesion to RGDS shows novel features compared with fibronectin. J Cell Biol 1987;105:507–15.
[4] Rajagopalan P, Marganski WA, Dembo M, Wong J. Traction stresses and morphology of 3t3 fibroblast cells on fibronectin- versus RGD-modified elastic substrata. Mater Res Soc Proc, in press.
[5] Cotrait M, Kreissler M, Hoflack J, Lehn JM, et al. Computational simulations of the conformational behaviour of the adhesive proteins Rgds fragment. J Computer-Aided Mol Des 1992;6:113–30.
[6] Hynes RO. Integrins: a family of cell surface receptors. Cell 1987;48:549–54.
[7] Leahy DJ. Implications of atomic-resolution structures for cell adhesion. Ann Rev Cell Developmental Biol 1997;13:363–93.
[8] Aota S, Nomizu M, Yamada KM. The short amino acid sequence Pro–His–Ser–Arg–Asn in human fibronectin enhances cell-adhesive function. J Biol Chem 1994;269:24756–61.
[9] Bowditch RD, Hariharan M, Tominna EF, Smith JW, et al. Identification of a novel integrin binding site in fibronectin. Differential utilization by beta 3 integrins. J Biol Chem 1994;269:10856–63.
[10] Ruoslahti E. RGD and other recognition sequences for integrins. Ann Rev Cell Developmental Biol 1996;12:697–715.
[11] Kimizuka F, Taguchi Y, Ohdate Y, Kawase Y, et al. Production and characterization of functional domains of human fibronectin expressed in *Escherichia coli*. J Biochem 1991;110:284–91.
[12] Altschul SF, Madden TL, Schaffer AA, Zhang JH, et al. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 1997;25:3389–402.
[13] Kabsch W, Sander C. Dictionary of protein secondary structure: pattern recognition of hydrogen-bonded and geometrical features. Biopolymers 1983;22:2577–637.
[14] Lee B, Richards FM. The interpretation of protein structures: estimation of static accessibility. J Mol Biol 1971;55:379–400.
[15] Connolly DT, Knight MB, Harakas NK, Wittwer AJ, et al. Determination of the number of endothelial cells in culture using an acid phosphatase assay. Anal Biochem 1986;152:136–40.
[16] Chen CS, Mrksich M, Huang S, Whitesides GM, et al. Geometric control of cell life and death. Science 1997;276:1425–8.
[17] Leahy DJ, Aukhil I, Erickson HP. 2.0 A crystal structure of a four-domain segment of human fibronectin encompassing the RGD loop and synergy region. Cell 1996;84:155–64.
[18] Redick SD, Settles DL, Briscoe G, Erickson HP. Defining fibronectin's cell adhesion synergy site by site-directed mutagenesis. J Cell Biol 2000;149:521–7.